Pharmacological Reports 69 (2017) 1315–1321

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Troglitazone, a PPAR-γ agonist, decreases LTC_4 concentration in mononuclear cells in patients with asthma

Emilia Luczak[a,1], Joanna Wieczfinska[a,1], Milena Sokolowska[a,b], Ewa Pniewska[a], Daria Luczynska[a], Rafał Pawliczak[a,*]

[a] Department of Immunopathology, Medical University of Lodz, Łódź, Poland
[b] Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland

ARTICLE INFO

Article history:
Received 22 November 2016
Received in revised form 6 May 2017
Accepted 11 May 2017
Available online 24 May 2017

Keywords:
Asthma
PPAR-γ
Troglitazone

ABSTRACT

Background: Asthma is an inflammatory disorder with multiple mediators involved in the inflammatory response. Despite several attempts, no new anti-inflammatory drugs have been registered for asthma treatment for several years. However, thiazolidinediones, peroxisome proliferator-activated receptor agonists, have demonstrated some anti-inflammatory properties in various experimental settings. The aim of this study was to assess the influence of troglitazone on LTC_4 and 15-HETE concentrations. It also evaluates TNF-induced eotaxin synthesis in peripheral blood mononuclear cells from 14 patients with mild asthma and 13 healthy controls.

Methods: PBMCs were isolated from the whole blood of the asthmatics and healthy subjects and pretreated with 0.1, 1 or 10 μM of Troglitazone. The cells were then exposed to 10^{-6} M calcium jonophore or 10 ng/ml TNF. The production and release of LTC_4, 15-HETE and eotaxin were then assessed.

Results: Troglitazone caused a dose-dependent inhibition in LTC_4 synthesis in both asthmatics and healthy subjects. Troglitazone did not influence 15-HETE or eotaxin production in either asthmatic patients or in healthy individuals.

Conclusion: Due to its inhibition of LTC_4 synthesis, troglitazone therapy is an interesting potential therapeutic approach in asthma and other LTC_4 related inflammatory disorders.

Introduction

Peroxisome proliferator-activated receptors (PPARs) were cloned for the first time in 1990 in rodent hepatic cells [1]. PPARs are ligand-activated transcription factors belonging to the nuclear hormone receptor (NR) superfamily, comprising steroid receptors, thyroid hormone receptors, and receptors for retinol and vitamins A and D [2]. PPARs regulate the expression of the genes involved in lipid and carbohydrate metabolism [3,4], as well as immunomodulators and regulators of inflammation. They take part in the etiology of atherosclerosis [4], obesity and diabetes [5] and aging [6].

There are three types of PPAR receptors, i.e. PPAR-α, PPAR-β/δ and PPAR-γ, and these are encoded by three different genes. All isoforms are characterized by similar structures and spatial conformations, but differ with regard to their tissue localization and function. The most exhaustively studied form is PPAR-γ. It has been identified as a transcription factor associated with adipocyte differentiation [7] and is known to be involved in apoptosis, cell cycle control [8] and the inhibition of cytokine secretion from inflammatory cells [9,10]. PPAR-γ is present in human and mouse monocytes/macrophages, and higher expression is observed during monocyte to macrophage differentiation and after cell activation by M-CSF or GM-CSF [11]. PPAR-γ agonists inhibit the secretion of IL-1β, IL-6, IL-10, IL-12 and TNF, suppress the expression of iNOS, COX and CCR2, and inhibit the function of other transcription factors (Ap-1, STAT, NF-κB) [12,13]. They also regulate the CD36 receptor [14]. In mouse T cells, PPAR-γ activation leads to decreased production of IL-2, IL-4, IL-5 and INF-γ [15]. PPAR-γ agonists decrease T cell proliferation [16] and can induce their apoptosis [17]. In addition, PPAR-γ inhibits the proliferation of B cells and enhances the response of B cells to antigen [18].

* Corresponding author.
E-mail address: rafal.pawliczak@csk.umed.lodz.pl (R. Pawliczak).
[1] Equally contributed.

http://dx.doi.org/10.1016/j.pharep.2017.05.006
1734-1140/

Thiazolidinediones (TZD – ciglitazone, rosiglitazone, troglitazone, pioglitazone) are synthetic ligands that bind to PPAR-γ [19,20]. After activation, PPAR-γ binds to the receptor for 9-*cis*-retinoic acid (RXR, NR2B) and, as a heterodimer, recognizes the 13-nucleotide Peroxisome Proliferator Response Element (PPRE) sequence (AGGTCA-N-AGGTCA) localized in the promoters of target genes. PPAR-γ can also act in a DNA-independent manner. It competes with other cofactor transcription factors, and can directly bind to NF-κB or NFAT to inhibit their action. The heterodimer represses genes expression by inhibition of the MAPK pathway [21].

Troglitazone was approved by the FDA in 1997 as a treatment for type II diabetes; however, it was withdrawn a few years later following reports of associated liver damage. Currently, rosiglitazone and pioglitazone are available as replacements. After the removal of the drug from the market, a few studies have assessed whether the hepatotoxicity caused by troglitazone could be associated with specific genetic variants. Watanabe et al. report that the presence of the combined *GSTT1* and *GSTM1* null genotype was a specific risk factor for the enhanced susceptibility to transaminase increase associated with troglitazone [22,23]. Oniki et al. suggest that the combination of *GSTM1* and *GSTT1* null genotypes might also be a risk factor for alcoholic mild liver dysfunction [24]. It still remains unclear whether side effects such as hepatotoxicity, myocardial infarct and heart failure are related to drug characteristics, such as the unique tocopherol side chain of troglitazone, or with other unknown mechanisms, and this has been the subject of considerable debate [22,25].

Asthma is characterized by chronic airway inflammation leading to bronchial hyperresponsiveness. T $CD4^+$ cells, mast cells and eosinophils are the main cells responsible for sustaining the inflammation. They produce a number of proinflammatory mediators, including cytokines, chemokines and eicosanoids, and play a role in the pathogenesis of asthma, together with neutrophils, monocytes/macrophages, dendritic cells, epithelial cells, smooth muscle cells and fibroblasts [26,27]. Chronic inflammation leads to airway remodeling, causing obturation associated with dyspnea, wheezing and decrease of pulmonary parameters [28].

Currently, asthma is incurable. Glucocorticoids, leukotriene receptor antagonists, short and long acting β-agonists, and anti-IgE allow the symptoms and airway inflammation to be controlled, but only to a certain extent, and chronic glucocorticoid therapy has been associated with adverse events. In addition, some refractory asthma phenotypes that concern patients with mild or moderate asthma are difficult to control because of recurrent episodes of severe dyspnea, and these often require hospitalization and oral glucocorticoid therapy [29–31].

A recent cohort study found that the troglitazone (TZD) used in diabetes treatment may decrease the number of asthma exacerbations [32]. In cultured HASM cells, TZD was shown to attenuate the TNF-α-induced production of eotaxin and MCP-1 expression. However, the extent to which TZDs can inhibit the effects of other proinflammatory stimuli and the release of other cytokines and chemokines remains to be established [33]. The main mediators of asthma are believed to be cysteinyl leukotrienes (CysLT) because of their potent constricting effects on bronchiolar smooth muscle [34,35]. Specific receptors of CysLT are known, and CysLT receptor inhibitors have been used to treat asthma [36–38].

A study on a mast cell line found troglitazone to strongly inhibit LTC_4 production induced by the type I allergy mechanism [39]. Many reports suggest that CysLT levels are increased in patients with asthma, and increase after allergen challenge, exercise challenge and with the severity of asthma, and decrease after treatment with a CysLT1 antagonist. CysLTs were also found to play a proinflammatory role following direct administration to the human airway [40–43]. However, despite a wealth of literature data in this area, few, if any, studies examine the effect of CysLTs on PBMC [44]. Changes in PBMC are easily noticeable, and can be easily employed in possible diagnostic strategies. In addition, the presence of systemic inflammation in chronic diseases also influences the function of PBMC [45,46].

The aim of our study is to assess the influence of troglitazone, a selective PPAR-γ agonist, on the release of the inflammatory mediators eotaxin, 15-(S)-HETE and leukotriene C_4 from the PBMCs of patients with mild asthma, compared to healthy individuals.

Materials and methods

Subjects

Fourteen patients suffering from non-severe asthma, and thirteen healthy volunteers participated in the study (Table 1). Asthma was diagnosed based on GINA 2015 criteria [47]; the subjects did not meet the American Thoracic Society Workshop on Refractory Asthma criteria for severe asthma.

The study was approved by the Local Ethical Committee, and informed consent was obtained from each participant before the study. The patients were not atopic, and were not being treated with oral GCS at least 6 months prior to the study. They were free from antihistamines and antileukotrienes; short-and long-acting β_2-agonists, and inhaled corticosteroids were withdrawn at least 24 h before the study visit. None of the participants were intolerant fo aspirin and other NSAIDs.

PBMC isolation

Blood samples were collected from the study participants. Peripheral blood mononuclear cells (PBMC) were isolated using Histopaque 1077 (Sigma-Aldrich, Saint Louis, MO, USA) density gradient centrifugation (400 × *g*, 35 min) and washed three times with PBS (250 × *g*, 10 min, 4 °C).

The cells were treated with troglitazone (TRO) – PPAR-γ agonist (Cayman Chemicals, Ann Arbor, MI, USA) at three concentrations 0.1 μM, 1 μM, and 10 μM, for 30 min. Control samples contained DMSO and untreated cells.

Each sample was then divided into two. One was treated with 10 ng/ml TNF for eight hours at room temperature (R&D Systems, Minneapolis, MN, USA) in order to stimulate eotaxin production; the other was treated with 1 μM calcium ionofore A23187 for 30 min at room temperature (Calbiochem, Darmstadt, Germany) in order to induce 15(S)-HETE and LTC_4 generation.

Table 1
Clinical data of patients with asthma and control subjects. FEV_1 and FVC values are presented as mean ± SE, other values are expressed as median (min-max) or percentage.

	Asthmatics	Healthy controls
N	14	13
Age [years]	36.5 (21–66)	26 (21–33)
gender f/m [%]	57/43	62/38
FEV_1 [L]	3.2 ± 0.7	n.a.
FEV_1 [%]	89.4 ± 12.0	n.a.
FVC [L]	4.5 ± 0.9	n.a.
Current smoker [%]	2	0
Inhaled GCS[μg/day][a]	378 (160–800)	n.a.
Long acting β_2- mimetics [μg/day]	50 (50–100)	n.a.
LTR_1receptor antagonists [mg/day]	10 (0–10)	n.a.
Short acting β_2- mimetics [μg/day]	200 (200–400)	n.a.
Antihistamines [mg/day]	3.5 (0–5)	n.a.

[a] Presented as μg of budesonide.

The samples were then centrifuged (250 × *g*, 15 min, 4 °C), and the supernatants were collected into new tubes. The samples were stored at −80 °C for the immunoassays.

Measuring concentrations of selected inflammatory mediators

ELISA was used to determine the concentrations of 15(S)-HETE, LTC_4 (Cayman Chemicals) and eotaxin (R&D Systems) in the samples. The sensitivity of the test was 5 pg/ml (eotaxin), 10 pg/ml (LTC_4) and 170 pg/ml (15(S)-HETE). The concentrations were measured in duplicate, and the measurements were standardized to the number of cells in each sample.

Statistical analysis

Data from the study was analyzed utilizing the Statistica software package (v.6.0; StatSoft Inc., Tulsa, OK, USA). The distribution of all examined variables was checked by the Kolmogorov-Smirnov and Lillieforse tests. The parametric Student's *t*-test, Cochran-Cox and one-way ANOVA tests were used to analyze normally-distributed data, while the non-parametric Mann-Whitney *U* test was used for non-normally distributed data. Data is expressed as mean ± SEM. A probability value less than 0.05 was considered statistically significant.

Results

The effect of troglitazone on the production of 15-(S)-HETE induced calcium ionophore by peripheral blood mononuclear cells

Troglitazone caused an insignificant decrease in 15- (S)- HETE production in both patients with asthma and healthy subjects (Fig. 1. $p > 0.05$). The average concentration of 15-(S)-HETE in a sample with calcium ionophore in the healthy controls group was higher than that measured in patients with bronchial asthma (Fig. 1. $p > 0.05$). No decreases in 15-(S)-HETE concentrations were observed after exposure to 0.1 μM, 1 μM or 10 μM troglitazone compared to control samples without troglitazone in both groups – patients in asthma and control group (Fig. 1. $p > 0.05$).

The effect of troglitazone on LTC_4 production induced by calcium ionophore in peripheral blood mononuclear cells

Higher levels of LTC_4 were observed in patients with asthma than the control group ($p < 0.05$) after A23187 stimulation. However, no decrease was observed in LTC_4 production in healthy subjects after the use of troglitazone as compared to the control samples (Fig. 2. $p < 0.05$). In patients with asthma, troglitazone significantly decreased LTC_4 production in a dose-response manner (Fig. 2. $p < 0.05$).

The effect of troglitazone on eotaxin production, induced by TNF in peripheral blood mononuclear cells

Both PBMCs from healthy subjects and bronchial asthma patients produced comparable concentrations of eotaxin in response to treatment with 0.1 μM, 1 μM and 10 μM troglitazone or without treatment. No changes in eotaxin production were observed in asthma patients after troglitazone administration (Fig. 3. $p > 0.05$). In addition, a stronger inhibitory effect can be observed at lower doses of troglitazone.

Discussion

Inflammation plays an important role in many airway diseases and is associated with the release of pro-inflammatory cytokines and oxygen free radicals from activated inflammatory cells such as neutrophils, eosinophils, monocytes and macrophages [48].

There is increasing evidence suggesting that PPAR-γ acts as an anti-inflammatory agent: it negatively regulates the expression of pro-inflammatory genes induced in response to inflammatory cell activation, but also inhibits cytokines, chemoattractants and cell survival factors involved in inflammation [48–50]. For this reason, the development of PPAR-γ agonists has been proposed as a novel anti-inflammatory target for inflammatory diseases such as asthma [51].

Although thiazolidinediones have potent anti-inflammatory activity and improve airway inflammation in murine models of

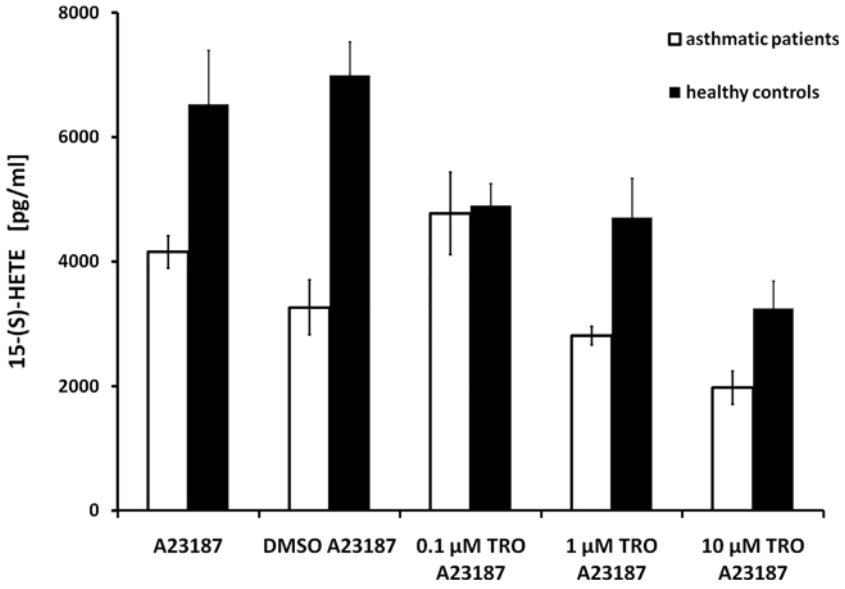

Fig. 1. Effect of troglitazone (TRO) to produce 15- (S)- HETE stimulated with calcium ionophore A23187 in patients with bronchial asthma and healthy subjects. Peripheral blood mononuclear cells were incubated with or without increasing concentrations of TRO for 30 min, then incubated with the calcium ionophore A23187 for 30 min. Concentration of 15-(S)- HETE in the supernatant of cells was measured by immunoassay. Data presented as mean concentration of 15- (S)-HETE (pg/ml) ± SD.

allergic inflammation [52,53], the impact of TZD on human asthma is not well known. The purpose of this study was to investigate the influence of TZD on eotaxin, 15-(S)-HETE and leukotriene C_4 secretion from the peripheral blood mononuclear cells of patients with mild asthma.

While PBMCs are thought to produce undetectable amounts of eotaxin, the cells tested were stimulated by TNF, which is known to highly induce eotaxin secretion [54,55]. Our study shows that eotaxin production was significantly lower in asthmatics than in healthy subjects: troglitazone did not influence TNF-induced eotaxin production in asthmatic patients. These results confirm those of a previous study by Desmet [56], which suggest that troglitazone does not inhibit the inflammatory response to TNF; however, eotaxin was not directly investigated in this study. Troglitazone significantly potentiates TNF-induced production of granulocyte/macrophage-colony-stimulating factor, interleukin 6 and/or interleukin 8 in epithelial cells. Our results contradict those of a previous study showing that troglitazone reduced the TNF-induced production of MCP-1 and eotaxin from HASM cells [33]. This difference is probably caused by differences in cell type and TNF concentration between the studies.

In contrast, a study by Nie et al. [57] found that PPAR-γ agonists, including troglitazone, inhibit TNF-induced eotaxin production on the transcriptional level. This was additively enhanced by glucocorticoids and β_2-agonists in human airway smooth muscle cells. As airway inflammation, particularly eosinophilia, is the main pathological feature of human asthma, these findings might suggest that troglitazone may act as a modulator of asthma and lung inflammation. It is also possible that the combination of PPAR-γ agonists with glucocorticoids or β_2-agonists enhances their overall anti-inflammatory effects, which may allow the doses of the individual drugs to be reduced, thus minimizing their side effects [57].

15-HETE is a known PPAR-γ ligand, and may increase PPAR-γ expression in epithelial cells [58]. Our findings indicate that troglitazone decreases 15-HETE concentrations in both asthma patients and the control group; therefore, PPAR-γ might be involved in the inflammatory cascade of asthma, and treatment with PPAR agonists may reduce airway inflammation. Surprisingly, in the present study, higher concentrations of 15-(S)-HETE were observed in healthy subjects than in asthmatics, which could be attributed to the use of small study groups, or the uneven response to the chosen concentrations of calcium ionophore.

Subbarayan et al. [59] report that normal intestinal epithelial cells show high levels of 15-(S)-HETE but low levels of PPAR-γ; this relationship was reversed in epithelial cancer cells. It has previously been demonstrated that incubation of peripheral blood leukocytes with aspirin resulted in significant increased 15-(S)-HETE generation in aspirin-sensitive patients, but not in aspirin-tolerant patients [60]. In addition, it did not influence LTC_4 generation in aspirin-tolerant nor aspirin-sensitive asthmatic patients.

In this study, asthmatic patients demonstrated higher LTC_4 concentrations than healthy controls. These concentrations were significantly lower after troglitazone exposure, with a dose-response within the 0.1–1 μM range. Furthermore, a significant dose-dependent decrease of LTC_4 concentration was observed in healthy subjects after troglitazone exposure. These results are similar to those obtained by Yamashita et al., which indicate that troglitazone suppresses the antigen-induced production of LTC_4 mast cells [39]. Liebhart et al. did not find that leukocytes have any ability to produce LTC_4 in response to various stimuli correlated with the magnitude of irreversible airway obstruction in asthmatic patients [61]. However, Yamashita et al. found that troglitazone inhibited the antigen-induced production of LTC_4 in mast cells [39].

Cysteinyl leukotrines appear to be one of many important mediators of inflammation in asthma. Several papers have suggested that their concentrations are not affected by the administration of inhaled glucocorticosteroids [62,63], which supports the use of the anti-leukotriene drugs in GINA armamentarium for the treatment of asthma. Hence, the use of troglitazone in treating asthma might decrease LTC_4 production to some degree. Further studies are needed to elucidate the mechanism involved in the decrease of LTC_4 production in asthmatics and in healthy individuals.

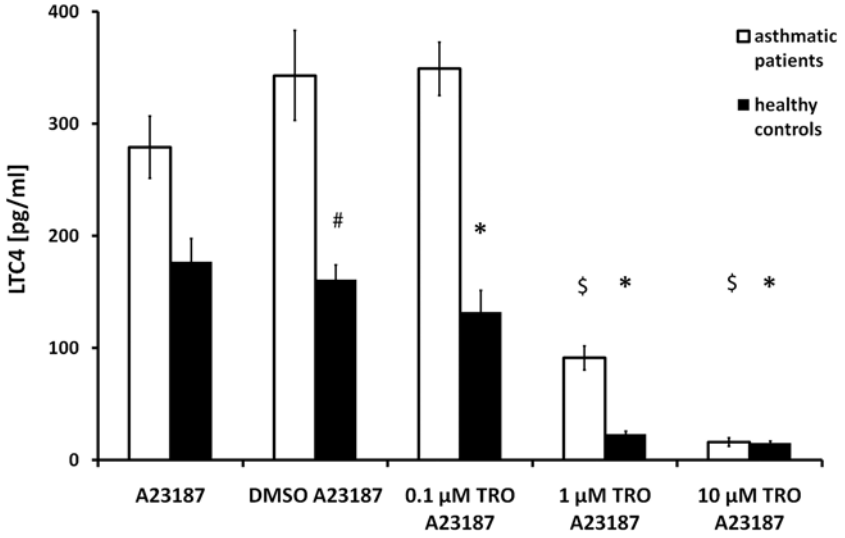

Fig. 2. Effect of troglitazone (TRO) on the production of LTC_4 stimulated with calcium ionophore A23187 in patients with bronchial asthma and healthy subjects. A23187–stimulated LTC_4 concentration was higher in asthmatic subjects (#, $p < 0.05$) as compared to healthy individuals. Troglitazone in the concentration range 0.1 μM – 10 μM ($p < 0.05$) significantly decreased the production of LTC_4 both in asthmatics (*$p < 0.05$, compared to healthy subjects) and in healthy individuals ($ $p < 0.05$, compared to DMSO sample) as assessed by ANOVA. Peripheral blood mononuclear cells were incubated with or without increasing concentrations of TRO for 30 min, then incubated with the calcium ionophore A23187 for 30 min. Concentration of LTC_4 in the supernatant of cells was measured by immunoassay. Data presented as mean LTC_4 concentrations (pg/ml) ± SD.

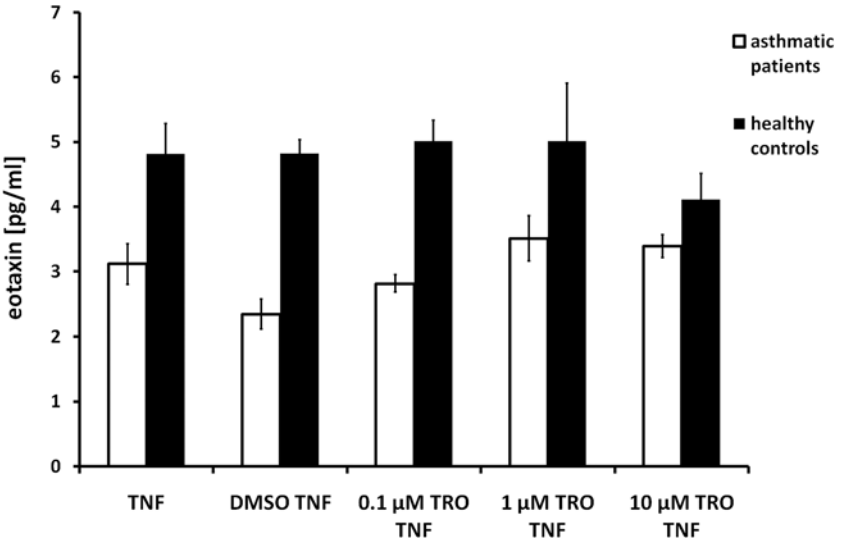

Fig. 3. Effect of troglitazone (TRO) in TNF- stimulated production of eotaxin in patients with bronchial asthma and healthy subjects. Peripheral blood mononuclear cells were incubated with or without increasing concentrations of TRO for 30 min, then incubated with TNF for 8 h. The concentration of eotaxin in the supernatant of cells was measured by immunoassay. Data presented as mean eotaxin levels (pg/ml) ± SD.

An important limitation of our study is its use of troglitazone, which has been shown to cause many adverse effects. However, besides troglitazone, rosiglitazone and pioglitazone also have particular warnings associated with congestive heart failure [22,64]. The toxicity of troglitazone has been attributed to the formation of a reactive quinone metabolite and to a quinone methide conjugate [65,66]. It is not known to what extent the liver cell can compensate or neutralize the oxidative damage from these compounds. In addition, the American Heart Association/American Diabetes Association consensus statement on TZD use supports their prescription to diabetic subjects at high risk of CVD based on their beneficial effects on vascular risk factors [67].

There is a need for new approaches to treating inflammatory diseases of the airways. Many groups have demonstrated the anti-inflammatory potential of synthetic and natural PPAR-γ agonists, both in cell-based assays and *in vitro* studies [48]. Studies have shown that PPAR-γ is expressed by human alveolar macrophages, and tests based on PPAR-γ agonists indicate that PPAR-γ plays an anti-inflammatory role in these cells by inhibiting cytokine production, increasing CD36 expression and enhancing the phagocytosis of apoptotic neutrophils, an essential process for the resolution of inflammation [48,68,69]. A recent study suggests that thiazolidinediones may decrease the number of asthma exacerbations in patients with diabetes [32]. Therefore, pharmacological targeting of PPAR-γ may be a new strategy for treating eosinophil-related diseases including bronchial asthma.

Conclusions

Our findings demonstrated that troglitazone decreases the release of LTC_4 in the PBMCs of both asthmatic patients and healthy controls. In addition, PBMC treatment with troglitazone inhibits LTC_4 synthesis in a dose-dependent manner in both groups.

Troglitazone appears to be an efficient inhibitor of LTC_4 synthesis, and so deserves further attention as part of a novel therapeutic approach to asthma and other LTC_4-related inflammatory disorders. Further studies utilizing currently registered TZD are needed to evaluate their anti-inflammatory properties.

Competing interests

The authors declare no conflicts of interest.

Funding

The study was supported by Medical University of Lodz (503/0-148-03/503-01-001 and 502-03/0-149-03/502-04-018)

References

[1] Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 1990;347(6294):645–50.
[2] Margolis RN, Christakos S. The nuclear receptor superfamily of steroid hormones and vitamin D gene regulation. An update. Ann N Y Acad Sci 2010;1192:208–14.
[3] Cha BS, Ciaraldi TP, Carter L, Nikoulina SE, Mudaliar S, Mukherjee R, et al. Peroxisome proliferator-activated receptor (PPAR) gamma and retinoid X receptor (RXR) agonists have complementary effects on glucose and lipid metabolism in human skeletal muscle. Diabetologia 2001;44(4):444–52.
[4] Li AC, Glass CK. PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis. J Lipid Res 2004;45 (12):2161–73.
[5] Freake HC. A genetic mutation in PPAR gamma is associated with enhanced fat cell differentiation: implications for human obesity. Nutr Rev 1999;57(5 Pt 1):154–6.
[6] Zhang R, Zheng F. PPAR-gamma and aging: one link through klotho? Kidney Int 2008;74(6):702–4.
[7] Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology 1994;135(2):798–800.
[8] Fujii D, Yoshida K, Tanabe K, Hihara J, Toge T. The ligands of peroxisome proliferator-activated receptor (PPAR) gamma inhibit growth of human esophageal carcinoma cells through induction of apoptosis and cell cycle arrest. Anticancer Res 2004;24(3a):1409–16.
[9] Yuan ZY, Liu Y, Zhang JJ, Kishimoto C, Wang YN, Ma AQ, et al. PPAR-gamma ligands inhibit the expression of inflammatory cytokines and attenuate autoimmune myocarditis. Chin Med J (Engl) 2004;117(8):1253–5.
[10] Ji JD, Cheon H, Jun JB, Choi SJ, Kim YR, Lee YH, et al. Effects of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) on the expression of inflammatory cytokines and apoptosis induction in rheumatoid synovial fibroblasts and monocytes. J Autoimmun 2001;17(3):215–21.
[11] Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, et al. Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci U S A 1998;95 (13):7614–9.

[12] Li M, Pascual G, Glass CK. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. Mol Cell Biol 2000;20(13):4699–707.
[13] Abdelrahman M, Sivarajah A, Thiemermann C. Beneficial effects of PPAR-gamma ligands in ischemia-reperfusion injury, inflammation and shock. Cardiovasc Res 2005;65(4):772–81.
[14] Feng J, Han J, Pearce SF, Silverstein RL, Gotto AM, Hajjar DP, et al. Induction of CD36 expression by oxidized LDL and IL-4 by a common signaling pathway dependent on protein kinase C and PPAR-gamma. J Lipid Res 2000;41(5):688–96.
[15] Mueller C, Weaver V, Vanden Heuvel JP, August A, Cantorna MT. Peroxisome proliferator-activated receptor gamma ligands attenuate immunological symptoms of experimental allergic asthma. Arch Biochem Biophys 2003;418(2):186–96.
[16] Clark RB, Bishop-Bailey D, Estrada-Hernandez T, Hla T, Puddington L, Padula SJ. The nuclear receptor PPAR gamma and immunoregulation: PPAR gamma mediates inhibition of helper T cell responses. J Immunol 2000;164(3):1364–71.
[17] Harris SG, Phipps RP. The nuclear receptor PPAR gamma is expressed by mouse T lymphocytes and PPAR gamma agonists induce apoptosis. Eur J Immunol 2001;31(4):1098–105.
[18] Padilla J, Kaur K, Harris SG, Phipps RP. PPAR-gamma-mediated regulation of normal and malignant B lineage cells. Ann N Y Acad Sci 2000;905:97–109.
[19] Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 1995;270(22):12953–6.
[20] Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. Diabetes 1998;47(4):507–14.
[21] Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, et al. International union of pharmacology: LXI. Eroxisome proliferator-activated receptors. Pharmacol Rev 2006;58(4):726–41.
[22] Della-Morte D, Palmirotta R, Rehni AK, Pastore D, Capuani B, Pacifici F, et al. Pharmacogenomics and pharmacogenetics of thiazolidinediones: role in diabetes and cardiovascular risk factors. Pharmacogenomics 2014;15(16):2063–82.
[23] Watanabe I, Tomita A, Shimizu M, Sugawara M, Yasumo H, Koishi R, et al. A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus. Clin Pharmacol Ther 2003;73(5):435–55.
[24] Oniki K, Ueda K, Hori M, Mihara S, Marubayashi T, Nakagawa K. Glutathione-S-transferase (GST) M1 null genotype and combined GSTM1 and GSTT1 null genotypes as a risk factor for alcoholic mild liver dysfunction. Clin Pharmacol Ther 2007;81(5):634–5.
[25] Tolman KG. Thiazolidinedione hepatotoxicity: a class effect? Int J Clin Pract Suppl 2000;113:29–34.
[26] Page C, O'Shaughnessy B, Barnes P. Pathogenesis of COPD and asthma. Handb Exp Pharmacol 2017;237:1–21.
[27] Guan Y, Jin X, Liu X, Huang Y, Wang M, Li X. Uncovering potential key genes associated with the pathogenesis of asthma: a microarray analysis of asthma-relevant tissues. Allergol Immunopathol (Madr) 2017;45(2):152–9.
[28] Pothen JJ, Poynter ME, Bates JH. A computational model of unresolved allergic inflammation in chronic asthma. Am J Physiol Lung Cell Mol Physiol 2015;308(4):L384–90.
[29] Lawani MA, Zongo F, Breton MC, Moisan J, Gregoire JP, Dorval E, et al. Factors associated with adherence to asthma treatment with inhaled corticosteroids: a cross-sectional exploratory study. J Asthma 2017;0.
[30] Axelsson M, Ekerljung L, Lundback B, Lotvall J. Personality and unachieved treatment goals related to poor adherence to asthma medication in a newly developed adherence questionnaire—a population-based study. Multidiscip Respir Med 2016;11:42.
[31] Boonpiyathad S, Sangasapaviliya A. Refractory asthma treatment is complicated by tracheobronchomalacia: case reports and review of the literature. Case Rep Med 2013;2013:735058.
[32] Rinne ST, Feemster LC, Collins BF, Au DH, Perkins M, Bryson CL, et al. Thiazolidinediones and the risk of asthma exacerbation among patients with diabetes: a cohort study. Allergy Asthma Clin Immunol 2014;10(1):34.
[33] Zhu M, Flynt L, Ghosh S, Mellema M, Banerjee A, Williams E, et al. Anti-inflammatory effects of thiazolidinediones in human airway smooth muscle cells. Am J Respir Cell Mol Biol 2011;45(1):111–9.
[34] Yamashita M. PPARalpha/gamma-independent effects of PPARalpha/gamma ligands on cysteinyl leukotriene production in mast cells. PPAR Res 2008;2008:293538.
[35] Sokolowska M, Wodz-Naskiewicz K, Cieslak M, Seta K, Bednarek AK, Pawliczak R. Variable expression of cysteinyl leukotriene type I receptor splice variants in asthmatic females with different promoter haplotypes. BMC Immunol 2009;10:63.
[36] Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, et al. Characterization of the human cysteinyl leukotriene 2 receptor. J Biol Chem 2000;275(39):30531–6.
[37] Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature 1999;399(6738):789–93.
[38] Dahlen B, Nizankowska E, Szczeklik A, Zetterstrom O, Bochenek G, Kumlin M, et al. Benefits from adding the 5-lipoxygenase inhibitor zileuton to conventional therapy in aspirin-intolerant asthmatics. Am J Respir Crit Care Med 1998;157(4 Pt 1):1187–94.
[39] Yamashita M, Kushihara M, Hirasawa N, Takasaki W, Takahagi H, Takayanagi M, et al. Inhibition by troglitazone of the antigen-induced production of leukotrienes in immunoglobulin E-sensitized RBL-2H3 cells. Br J Pharmacol 2000;129(2):367–73.
[40] Laidlaw TM, Boyce JA. Cysteinyl leukotriene receptors, old and new; implications for asthma. Clin Exp Allergy 2012;42(9):1313–20.
[41] Okunishi K, Peters-Golden M. Leukotrienes and airway inflammation. Biochim Biophys Acta 2011;1810(11):1096–102.
[42] Zhu J, Qiu YS, Figueroa DJ, Bandi V, Galczenski H, Hamada K, et al. Localization and upregulation of cysteinyl leukotriene-1 receptor in asthmatic bronchial mucosa. Am J Respir Cell Mol Biol 2005;33(6):531–40.
[43] Wenzel SE. The role of leukotrienes in asthma. Prostaglandins Leukot Essent Fatty Acids 2003;69(2–3):145–55.
[44] Singh RK, Tandon R, Dastidar SG, Ray A. A review on leukotrienes and their receptors with reference to asthma. J Asthma 2013;50(9):922–31.
[45] Pniewska E, Sokolowska M, Kuprys-Lipinska I, Kacprzak D, Kuna P, Pawliczak R. Exacerbating factors induce different gene expression profiles in peripheral blood mononuclear cells from asthmatics, patients with chronic obstructive pulmonary disease and healthy subjects. Int Arch Allergy Immunol 2014;165(4):229–43.
[46] Aldonyte R, Jansson L, Piitulainen E, Janciauskiene S. Circulating monocytes from healthy individuals and COPD patients. Respir Res 2003;4:11.
[47] Doherty TA, Soroosh P, Broide DH, Croft M. CD4+ cells are required for chronic eosinophilic lung inflammation but not airway remodeling. Am J Physiol Lung Cell Mol Physiol 2009;296(2):L229–35.
[48] Belvisi MG, Hele DJ, Birrell MA. Peroxisome proliferator-activated receptor gamma agonists as therapy for chronic airway inflammation. Eur J Pharmacol 2006;533(1–3):101–9.
[49] von Knethen A, Brune B. PPARgamma-an important regulator of monocyte/macrophage function. Arch Immunol Ther Exp (Warsz) 2003;51(4):219–26.
[50] Patel HJ, Belvisi MG, Bishop-Bailey D, Yacoub MH, Mitchell JA. Activation of peroxisome proliferator-activated receptors in human airway smooth muscle cells has a superior anti-inflammatory profile to corticosteroids: relevance for chronic obstructive pulmonary disease therapy. J Immunol 2003;170(5):2663–9.
[51] Serhan CN, Devchand PR. Novel antiinflammatory targets for asthma. A role for PPARgamma? Am J Respir Cell Mol Biol 2001;24(6):658–61.
[52] Zhao Y, Huang Y, He J, Li C, Deng W, Ran X, et al. Rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates airway inflammation by inhibiting the proliferation of effector T cells in a murine model of neutrophilic asthma. Immunol Lett 2014;157(1–2):9–15.
[53] Park YS, Lillehoj EP, Kato K, Park CS, Kim KC. PPARgamma inhibits airway epithelial cell inflammatory response through a MUC1-dependent mechanism. Am J Physiol Lung Cell Mol Physiol 2012;302(7):L679–87.
[54] Olsson S, Cagnoni F, Dignetti P, Melioli G, Canonica GW. Low concentrations of cytokines produced by allergen-stimulated peripheral blood mononuclear cells have potent effects on nasal polyp-derived fibroblasts. Clin Exp Immunol 2003;132(2):254–60.
[55] Wong CK, Zhang JP, Ip WK, Lam CW. Activation of p38 mitogen-activated protein kinase and nuclear factor-kappaB in tumour necrosis factor-induced eotaxin release of human eosinophils. Clin Exp Immunol 2002;128(3):483–9.
[56] Desmet C, Warzee B, Gosset P, Melotte D, Rongvaux A, Gillet L, et al. Pro-inflammatory properties for thiazolidinediones. Biochem Pharmacol 2005;69(2):255–65.
[57] Nie M, Corbett L, Knox AJ, Pang L. Differential regulation of chemokine expression by peroxisome proliferator-activated receptor gamma agonists: interactions with glucocorticoids and beta2-agonists. J Biol Chem 2005;280(4):2550–61.
[58] Ban K, Sprunt JM, Martin S, Yang P, Kozar RA. Glutamine activates peroxisome proliferator-activated receptor-gamma in intestinal epithelial cells via 15-S-HETE and 13-OXO-ODE: a novel mechanism. Am J Physiol Gastrointest Liver Physiol 2011;301(3):G547–54.
[59] Subbarayan V, Xu XC, Kim J, Yang P, Hoque A, Sabichi AL, et al. Inverse relationship between 15-lipoxygenase-2 and PPAR-gamma gene expression in normal epithelia compared with tumor epithelia. Neoplasia 2005;7(3):280–93.
[60] Kowalski ML, Ptasinska A, Bienkiewicz B, Pawliczak R, DuBuske L. Differential effects of aspirin and misoprostol on 15-hydroxyeicosatetraenoic acid generation by leukocytes from aspirin-sensitive asthmatic patients. J Allergy Clin Immunol 2003;112(3):505–12.
[61] Liebhart J, Medrala W, Gladysz U, Barg W, Liebhart E, Dobek R, et al. Production of leukotriene C4 by peripheral blood leukocytes stimulated with anti-fcepsilonRI antibody, PMA, and fMLP does not correlate with irreversible airway obstruction in asthmatics. J Investig Allergol Clin Immunol 2007;17(1):1–5.
[62] Dworski R, Fitzgerald GA, Oates JA, Sheller JR. Effect of oral prednisone on airway inflammatory mediators in atopic asthma. Am J Respir Crit Care Med 1994;149(4 Pt 1):953–9.
[63] Bush A. Montelukast in paediatric asthma: where we are now and what still needs to be done? Paediatr Respir Rev 2015;16(2):97–100.
[64] Filion KB, Joseph L, Boivin JF, Suissa S, Brophy JM. Thiazolidinediones and the risk of incident congestive heart failure among patients with type 2 diabetes mellitus. Pharmacoepidemiol Drug Saf 2011;20(8):785–96.

[65] Kassahun K, Pearson PG, Tang W, McIntosh I, Leung K, Elmore C, et al. Studies on the metabolism of troglitazone to reactive intermediates in vitro and in vivo. Evidence for novel biotransformation pathways involving quinone methide formation and thiazolidinedione ring scission. Chem Res Toxicol 2001;14(1):62–70.
[66] Haskins JR, Rowse P, Rahbari R, de la Iglesia FA. Thiazolidinedione toxicity to isolated hepatocytes revealed by coherent multiprobe fluorescence microscopy and correlated with multiparameter flow cytometry of peripheral leukocytes. Arch Toxicol 2001;75(7):425–38.
[67] Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, Sherwin R, et al. Management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diab Care 2006;29(8):1963–72.
[68] Asada K, Sasaki S, Suda T, Chida K, Nakamura H. Antiinflammatory roles of peroxisome proliferator-activated receptor gamma in human alveolar macrophages. Am J Respir Crit Care Med 2004;169(2):195–200.
[69] Sokolowska M, Borowiec M, Ptasinska A, Cieslak M, Shelhamer JH, Kowalski ML, et al. 85-kDa cytosolic phospholipase A2 group IValpha gene promoter polymorphisms in patients with severe asthma: a gene expression and case-control study. Clin Exp Immunol 2007;150(1):124–31.