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A PPAR-α agonist protects the non-adrenergic, non-cholinergic inhibitory system of guinea pig trachea from the effect of inhaled ammonium persulphate: a pilot study

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ABSTRACT. **Aim of the study.** ***Inhaled ammonium persulphate (AP) reduces non adrenergic, non cholinergic (NANC) relaxation in the guinea pig trachea, as a part of its inflammatory effects. Peroxisome Proliferator-Activated Receptor (PPAR) stimulation has shown anti-inflammatory properties. This study aimed at evaluating whether the PPAR-α agonist WY 14643 can prevent the reduction in NANC relaxation caused by inhaled AP in the guinea pig trachea.***
Materials and Methods. ***Four groups of ten male guinea pigs were treated for three weeks with inhaled AP (10 mg/m^3, 30 min per day, group A), saline (group B), AP and WY 14643 (0.36 µM/die, per os, group C), and AP, WY 14643 and the PPAR-α antagonist GW 6471 (0.36 µM/die, per os, group D). NANC relaxations to electrical field stimulation (EFS) at 3 Hz were evaluated in whole tracheal segments as intraluminal pressure changes.***
Results. ***The tracheal NANC relaxations were reduced by 90.3% in group A, as compared to group B. In group C, they were reduced by only 22.2%. In group D, they were reduced by 92.6 %. PPAR-α receptors were detected in inhibitory nerve fibers within the trachea as shown by immonohistochemical analysis.***
Conclusions. ***The PPAR-α agonist WY 14643 protects the NANC inhibitory system of the guinea pig trachea from the effect of inhaled ammonium persulphate and its protective effect is antagonized by GW 6471. PPAR-α might be exploited as a pharmacological target in asthma therapy.***

Key words: *PPAR-α, Ammonium persulphate, Inhibitory NANC innervation, Airways, Occupational asthma.*

RIASSUNTO. Un agonista PPAR-α protegge il sistema inibitorio non adrenergico, non colinergico della trachea di cavia dall'effetto dell'inalazione di ammonio persolfato: uno studio pilota.
Scopo dello studio. **L'inalazione di ammonio persolfato (AP) riduce il rilasciamento non adrenergico, non colinergico (NANC) nella trachea di cavia, come parte dei suoi effetti infiammatori. La stimolazione dei Peroxisome Proliferator-Activated Receptor (PPAR) ha mostrato di possedere effetti antiinfiammatori. Questo studio ha avuto lo scopo di valutare se l'agonista per i PPAR-α WY 14643 sia in grado di prevenire la riduzione del rilasciamento NANC causata dall'inalazione di AP nella trachea di cavia.**
Materiali e metodi. **Quattro gruppi di dieci cavie maschio sono stati trattati con l'inalazione di AP (10 mg/m^3, 30 minuti al giorno, gruppo A), con soluzione fisiologica (gruppo B), con AP e WY 14643 (0.36 µM/die, per os, gruppo C), e con AP, WY 14643 e con l'antagonista PPAR-α GW 6471 (0.36 µM/die, per os, group D). I rilasciamenti NANC indotti dalla stimolazione con campo elettrico, alla frequenza di 3 Hz, sono stati valutati**

Introduction

Several etiological agents of occupational asthma, including persulphates, are irritants at high concentrations, whereas they behave as sensitizing agents in the case of chronic exposure at low concentrations (1). It has been estimated that persulphate-induced asthma may represent up to 4% of all occupational asthma cases (2). In particular, persulphates are the major agents causing occupational asthma in hairdressers (3). Humoral and cellular immune-mediated mechanisms are thought to be involved, but direct irritation of the airway mucosa and dysregulation of

ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AECOPD	acute exacerbation of chronic obstructive pulmonary disease
ALI	acute lung injury
AP	ammonium persulphate
AUC	area under the curve
CNS	central nervous system
CO	carbon monoxide
DMSO	dimethylsulfoxide
EFS	electrical field stimulation
FEV1	forced expiratory volume in the first second
FVC	forced vital capacity
LPS	lipopolysaccharide
MEF 25	maximal expiratory flow at 75% of forced vital capacity being exhaled
MEF75	maximal expiratory flow at 25% of forced vital capacity being exhaled
NANC	non adrenergic, non cholinergic
NANCi	non adrenergic, non cholinergic inhibitory
NF	neurofilament
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
PPAR	peroxisome proliferator-activated receptor
PPAR-α(-/-)	PPAR-α knockout
TLV	threshold limit value
TNF-α	tumor necrosis factor-α
TTX	tetrodotoxin
VC MAX	maximal vital capacity
WT	wild-type

come variazioni di pressione intraluminale in segmenti tracheali interi.
Risultati. **I rilasciamenti NANC tracheali erano ridotti del 90.3% nel gruppo A, a confronto con il gruppo B. Nel gruppo C essi erano ridotti solo del 22.2%. Nel gruppo D i rilasciamenti erano ridotti del 92.6%. È stata rilevata la presenza di recettori PPAR-α nelle fibre nervosa inibitorie intrinseche della trachea, mediante analisi immunoistochimica.**
Conclusioni. **L'agonista per i PPAR-α WY 14643 protegge il sistema NANC inibitorio della tracea di cavia dall'effetto dell'inalazione di ammonio persolfato e il suo effetto protettivo è antagonizzato dall'antagonista specifico GW 6471. I PPAR-α potrebbero essere sfruttati come bersaglio farmacologico nella terapia dell'asma.**

Parole chiave: PPAR-α, ammonio persolfato, innervazione NANC inibitoria, vie aeree, asma professionale.

the intrinsic innervation can contribute substantially to the pathogenesis of the disease (4,5). Especially the latter processes can play a main role in non-immune-mediated manifestations, therefore in the pathogenesis of non-allergic occupational asthma, also called irritant-induced occupational asthma, consisting in a reactive airways dysfunction syndrome (RADS), a reactive upper airways dysfunction syndrome (RUDS) or in sub-acute irritant-induced occupational asthma (6).

Two studies by our group showed that ammonium persulphate inhalation at high concentrations impairs the nervous non adrenergic, non cholinergic inhibitory (NANCi) control in the guinea pig trachea (7), without affecting the contribution of single neurotransmitters, mainly nitric oxide (NO) and carbon monoxide (CO) (8) to nerve-mediated relaxation. A dysregulation of the intrinsic innervation, has been suggested as a major focus for the research on the pathogenesis of different forms of asthma (9-12). An altered NO-mediated inhibitory response as a cause of airway dysfunction due to a chronic exposure to oxidizing substances has been firstly hypothesized (13) and subsequently some evidence has been obtained. In particular, a study concluding that airways reactivity to methacholine in C57BL/6 mice treated with fenofibrate is dose and time dependently decreased, showed that, after administration of a NOS inhibitor, both vehicle and fenofibrate treated mice exhibited similar reactivity to methacholine, suggesting that NO mediates fenofibrate-induced decrease in airways reactivity (14).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the steroid, thyroid and retinoid nuclear receptor superfamily (15). They are activated by fatty acids and their derivatives. Different isotypes of PPARs (alpha, beta/delta and gamma) have distinct physiological functions, depending on their different ligand activation profiles and tissue distribution (16). The overall ability of PPAR agonists to modulate inflammatory processes is still awaiting to be fully assessed. While some PPAR-gamma (PPAR-γ) agonists have already been proposed as potential therapeutic agents for chronic airway inflammation (16-21), the potential of PPAR-alpha (PPAR-α) agonists as possible modulators of airway inflammation has been less investigated so far (22,23). A study showed that the activation of PPAR-α by WY 14643, a low potent but highly selective agonist, attenuated lipopolysaccharide (LPS)-mediated acute lung injury (ALI), as evidenced by the inhibition of the increases in pro-inflammatory cytokines and nitroxidative stress levels in the lung tissues of wild-type (WT) mice, but not in the corresponding age-matched PPAR-α knockout (PPAR-α(-/-)) mice (24).

An experimental study on alveolar epithelial cells isolated from WT and PPAR-α(-/-)) mice after 14 days of feeding with chow enriched with or without fibrate compound WY-14643, concluded that PPAR-α activation by *in vivo* exposure to fibrate compounds reduced the inflammatory response assessed as concentration of tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-2, and thromboxane B2 (25). Another experimental study on Pseudomonas aeruginosa (PA) infection, inducing lung inflammation *in vivo*, focused on evaluating the involvement of TLR4 and PPARα receptors. TLR4 (-/-) and PPAR-α (-/-) mice showed a worsened pathophysiology of PA lung disease compared with WT mice. The authors concluded that these receptors would mediate the proinflammatory responses to PA infections; in particular PPARα receptors are needed to prevent an excessive inflammatory response, as in the control of the inflammasome complex NLP3 activation (26).

One more study using an *in vitro* and *in vivo* model of ALI, and blood samples of patients with acute respiratory distress syndrome due to LPS and donors' controls, evidenced that PPAR-α is essential for the recovery of lung function by suppressing the level of TGF β1 (27).

Moreover, a review about the effect of PPARs agonists on prevention and treatment of lung cancer points out that PPAR-α may play a two-pronged role; complete absence of PPAR-α expression allows tumor clearance by the host's immune system, while agonist-induced stimulation of PPAR-α prohibits the exaggerated inflammatory and angiogenic responses of the host, that can favor tumor development (28).

Fenofibrate has been shown to suppress allergen induced airway inflammation by switching orientation of the immune response to allergen towards a Th1 profile (29). Besides infiltrated inflammatory cells, various structural and resident cells all throughout the airways express PPAR-α and PPAR-γ. that could contribute to limit airway inflammation and remodeling (30).

NANCi neurons, that have been shown to be functionally impaired in the presence of an inflammatory process (31), could also express PPARs, like CNS neurons (32). Evidence that PPAR-α activation by fenofibrate attenuates NO-mediated neuronal and axonal damage has been provided in the CNS (33). Whether PPAR-α stimulation can also protect airway NANCi neurons remains unsettled. A suggestion of this protective role could be given by a recent study. Peripheral blood samples from patients with severe stage of chronic obstructive pulmonary disease (AECOPD) receiving conventional therapy with fenofibrate, patients with severe AECOPD and controls were collected, lymphocytes isolated and enzyme immunode-

tection used to quantify the expression of TNF-α. Each participant underwent a spirography with broncholytic breakdown. Fenofibrate, in addition to the direct immunomodulating effect (increase in TNF-α levels), significantly improved pulmonary function (assessed as FVC, FEV1/FVC, MEF 75, MEF 25 and VC MAX) (34).

A recent review highlights the still current choice of guinea pigs as respiratory animal model compared to mice, especially focusing on the pharmacological aspects of the disease and the anatomical and physiological similarities to human (airway branching, neurophysiology, pulmonary circulation and smooth muscle distribution, as well as mast cell localization and mediator secretion). Acute allergen response, subacute effects, cough reflex, neural control, remodeling after chronic appropriate exposures mimic quite accurately their human counterpart, allowing several interspecies evaluations and explaining why this model has been extensively used in the last decades (35-39).

WY 1463 was chosen as PPAR-α agonist because it has a favourable combination of potency and receptor selectivity, as shown by its EC_{50} of 0.63 μM for PPAR-α and 32 μM for PPAR-γ.

This randomized double-blind parallel experimental study aimed at evaluating whether the PPAR-α agonist WY 14643 can prevent the reduction in NANC relaxation caused by inhaled ammonium persulphate (AP) in the guinea pig trachea. It aims contingently at providing a better insight in the neuronal mechanisms involved in asthma development in the occupational setting and a possible pharmacological target for treatment.

Materials and Methods

Animals

We used male albino guinea pigs weighing 500-600 g (Bettinardi, Momo, Italy). Animals were housed in standard facilities, at constant temperature (21 ± 1°C), at relative humidity of (50-55%), and alternating 12 hours light and 12 hours dark cycles. We provided them with food and water ad libitum. We followed the European Union Directive 2010/63/EU and National Institutes of Health recommendations for the humane use of animals, also in accordance with the principles of the Declaration of Helsinki. All experimental procedures were reviewed and approved by the Animal Use Committee of the University of Pavia, Italy. The number of animals used was kept to the minimum necessary for a meaningful interpretation of the data and animal discomfort was kept to the minimum. For functional studies, animals were killed by cervical dislocation and rapid exsanguination.

Treatments

The animals were randomly assigned to a control group or one of three treatment groups in a double-blind manner, each of the four groups including 10 male guinea pigs. They were subjected to the inhalation of different aerosols during 30 min for 5 consecutive days, 3 weeks in a row. Two groups were also administered with oral treatments. The guinea pigs in the control group (group B) were exposed at an isotonic saline aerosol (0.9% NaCl). The subjects in the first treatment group (group A) were exposed by inhalation to an aerosolized aqueous solution of ammonium persulphate (AP) at irritating concentrations, (10 mg/m^3), higher than the Threshold Limit Value (TLV) of 0.1 mg/m^3 (American Conference of Governmental Industrial Hygienists - ACGIH).

The ammonium persulphate threshold limit value (TLV) proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) is 0.1 mg/m^3, while a NOAEL of 1 mg/m^3 for symptoms of lung inflammation, edema or loss of body weight is reported from an inhalation study in animals with ammonium persulfate (ACGIH, 2018). We chose a concentration that was 100 fold the TLV time weighted average, aiming to elicit both sensitizing and irritant mechanisms, avoiding approaching the value indicated by the Organisation of Economic Cooperation and Development Guidelines LC_{50}: >2,950 mg/m^3 (4 hours, rats, acute) in 2005.

The second treatment group (group C) underwent exposure to AP and to WY 14643 (0.36 μM/die, per os), according to the same time schedule. The last group (group D) underwent AP solution inhalation, WY 14643 per os and the PPAR-α antagonist GW 6471 (0.36 μM/die, per os) during the 3 weeks. The aerosol was generated by an ultrasonic nebulizer (DeVilbiss Ultra Neb 2000, particle size range 0.5-5 μm, mean 2.8 μm, with an output settable from 0 to 7.5 ml/min solution), connected to a plexiglas exposure chamber (5 liters) with continuous air flow. Oral WY 14643 was administered on daily one hour before exposure to inhaled AP and GW 6471 (when needed) was administered together with the agonist, The doses of WY 14643 and GW 6471 were chosen in order to reach in the animal's body a concentration approaching the EC_{50} of the agonist.

Functional Studies

Twelve hours after the last exposure, the trachea was excised and transferred to a Petri dish containing oxygenated standard Tyrode solution (95% O_2 and 5% CO_2). A 3 cm-long tracheal tube was prepared by gently removing the mucosa (40), to avoid the electrical stimulation of epithelial cells, to avoid the release of substantial amounts of prostanoids, ATP and NO that may alter the nerve-mediated response to electrical field stimulation (EFS) (40). Since this technique allows a histologically proved total mucosa ablation, the use of prostanoid synthesis inhibitors (e.g. indomethacin) was no longer needed. We cannulated the tracheal segment at each extremity by means of two polyvinyl chloride tubes (outer diameter, 2.0 mm; inner diameter, 1.35 mm), and kept it horizontally in a 10 ml organ bath containing Tyrode solution, at 37°C, bubbled with a mixture of 95% O_2 and 5% CO_2. The preparation was flushed intraluminally with a peristaltic pump delivering Tyrode solution at 0.4 ml min^{-1} for 30 min. Then, one end of the preparation was occluded and the other one was connected to a pressure transducer for intraluminal pressure measurement. Signals were

recorded using a PowerLab data acquisition system (ADInstruments Ltd., Crowborough, UK), and analyzed using PowerLab Chart v4.1.1 software.

After one hour of equilibration, the tracheal segment was stimulated by means of two platinum electrodes placed in parallel, 1 cm apart, and connected to an electrical stimulator (MARB ST 87). Trains of rectangular pulses (0.5 ms duration, 0.3-30 Hz frequency at 60 V) were delivered for 5 s at 10 min intervals. EFS induced NANC relaxations were measured as reduction in the intratracheal pressure. They were characterized by an initial fast response (peak response) followed by a late slow recovery of the tone up to the basal value (late response). We evaluated the overall inhibitory response as area under the curve (AUC), calculated as the integral from baseline for each response [Pascal (Pa)·s]. We chose to study the relaxant responses at 3 Hz because they were the closest to 50% of the maximal electrically induced relaxation at 30 Hz. All the experiments were carried out under NANC conditions, in the presence of hyoscine (1 μM) to block muscarinic acetylcholine receptors, piperoxan (1 μM) and propranolol (1 μM) to block α-and β-adrenoceptors.

The neurotoxin TTX (1 μM) was given in a subset of experiments to ensure that EFS evoked inhibitory responses were neurogenic.

Immunohistochemistry

Segments of excised trachea (total length 3 cm) were quickly dissected and processed as described previously (7). They were obtained from the control group (group B), in order to avoiding interference by inhaled or oral treatments. Briefly, specimens were fixed in 70-100% ethanol, rinsed in xylene and embedded in paraffin. The trachea samples were then cut into 5 μm thick sections by a microtome and processed for triple-labelling indirect immunofluorescence (7). Before immunostaining, sections were deparaffined in xylene, then gradually rehydrated in 100-70% ethanol scale solutions, rinsed in phosphate buffer (PB) and incubated in 10% normal donkey serum with 0.3% glycine for 1 h at room temperature in order to reduce non-specific binding. Trachea slices were incubated overnight at 4°C with the following mixtures containing rabbit polyclonal anti PPAR-α (1:100; Abcam, AB8934), sheep polyclonal anti-neuronal nitric oxide synthase (nNOS, 1:200, Santa Cruz Biotechnology, AB6175) and mouse monoclonal anti-neurofilament (NF) (1:500; Sigma-Aldrich Chemie, N0142). Following washing, slices were incubated for 1 h at room temperature with a mixture of affinity-purified donkey anti-rabbit IgG coupled with fluorescein isothiocyanate (FITC; 1:400), together with donkey anti-sheep IgG conjugated with tetramethyl-rhodamine isothiocyanate (TRITC; 1:400) and donkey anti-mouse IgG conjugated with aminomethyl-coumarin-acetate (AMCA; 1:100), washed again and coverslipped with glycerol in PB (9:1). All the secondary antisera were purchased from Jackson ImmunoResearch. Fluorescent images were acquired with a Zeiss Axioplan (Zeiss, Thornwood, N.Y., USA) microscope, equipped with appropriate filter cubes to discriminate the different fluorochrome applied. Images were recorded with a Polaroid DMC digital photocamera (Polaroid, Cambridge, Mass., USA) and DMCV2 software. Images were further processed using Corel Photo Paint and Corel Draw software programs (Corel, Milan, Italy). The specificity of the PPAR-α antibody was assessed in control experiments, performed by omitting the primary antibodies or by replacing them with a non-immune rabbit serum. Furthermore, we confirmed the absence of cross-reactivity among either the primary or secondary antibodies by performing experiments with primary antibodies being incubated separately, together with the corresponding secondary antisera.

Statistical Analysis

Data were analyzed as raw data (AUC), expressed as the mean ± s.e.m. of the percent residual response after treatment compared to control response (100%). Statistical analysis was performed using Student's t-test for paired or unpaired data and, when needed, analysis of variance, followed by Bonferroni's test for multiple comparisons. The data and statistical analysis fulfill the recommendations on experimental design and analysis in pharmacology (41). A p-value less than 0.05 was considered statistically significant.

Drugs

Hyoscine, isoprenaline, propranolol, tetrodotoxin (TTX) (all purchased from Sigma-Aldrich, St. Louis, MO, USA) and piperoxan (Rhône-Poulenc, Courbevoie, France) were dissolved in distilled water.

WY 14643 and GW 6471 (purchased from Tocris Bioscience, Ellisville, Mo, USA) were prepared by first dissolving it in dimethylsulfoxide (DMSO) and then diluting in distilled water.

Results

Under NANC conditions, EFS at 0.3 - 30 Hz induced relaxant responses, consisting of an initial fast phase (peak response) followed by a late slow response up to the recovery of the basal tone, which were reproducible over a 7 hours' time interval and were abolished by 1 μM TTX (n=4). EFS-induced relaxations were frequency-dependent and sub-maximal compared with the response evoked by 10 μM isoprenaline (peak response: 45% and 67%, at 3 and 10 Hz, respectively; n=10). The excitatory responses to nerve stimulation at 1, 3, 5, 10 Hz were frequency-dependent. These responses were abolished by either hyoscine or TTX (1 μM each; n=4) indicating a cholinergic nerve-mediated contraction (31).

In isolated tracheal segments from exposed animals, the tracheal NANC relaxations to 3 Hz stimulation were reduced by 90.3 ± 1.5% in group A (animals only exposed to inhaled AP), as compared to group B (control group, animals treated with inhaled saline) ($P < 0.01$). In group C (animals treated with inhaled AP and oral WY 14643), they were reduced by only 22.2 ± 12.8% ($P < 0.05$ vs.

group A). In group D (animals treated with inhaled AP and oral WY 14643 and GW 6471), they were reduced by 92.6 ± 0.5%, a result similar to that of group A, showing that the protective effect of the PPAR-α agonist was abolished by the antagonist (Fig. 1).

PPAR-α immunolabeling (Fig. 2B) was identified in NF positive nerve fibers or bundles (Fig. 2A) running through the smooth muscle of the guinea pig trachea. Some of the PPAR-α expressing fibers colocalized with nNOS immunoreactivity (Fig. 2C and 2D). PPAR-α immunolabeling was exclusively observed in neural structures and not in epithelial cells. Since the samples were obtained from the control group, as stated before, immunohistochemistry aimed only at confirming the presence of PPAR-α receptors in the same nerve fibers involved in NANC relaxation and did not explore the possible effects of inhaled AP or of the administered drugs on the expression of PPAR-α receptors.

Discussion

The present study confirms our previous observations (31) that inhalation of AP at high concentrations severely impairs the nervous NANCi control in the guinea pig isolated trachea. The PPAR-α agonist WY 14643 protects the NANCi system of the guinea pig trachea from the effect of inhaled ammonium persulphate. The immunohistochemical study showed colocalization of PPAR-α with nNOS in nerve fibers, thus indicating that they originate from inhibitory neurons. Specificity of PPAR-α immunoreactivity was demonstrated by the absence of labelling in sections in which the primary antibodies were omitted or substituted with non-immune serum.

The mechanisms underlying persulphate-induced asthma include immunologic humoral and cellular events. IgEs are known to participate in the sensitizing

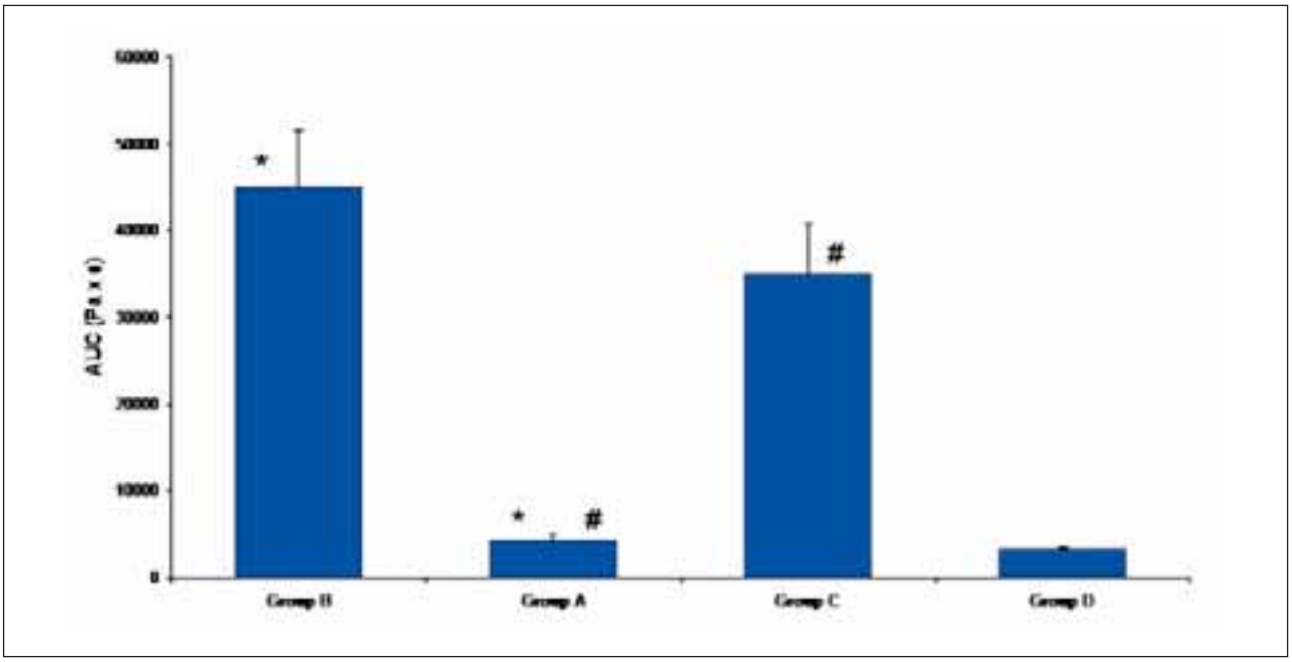

Figure 1. ***EFS-induced NANC relaxations evoked by 3 Hz in isolated whole trachea were evaluated as AUC of the overall inhibitory response (including both the fast and the slow component) in the four groups of animals. Group A: inhaled AP. Group B: inhaled saline (control group). Group C: inhaled AP + WY 14643. Group D: inhaled AP + WY 14643 + GW 6471. Data represent mean ± s.e.m. of 10 experiments***

**** P < 0.01. # P < 0.05***

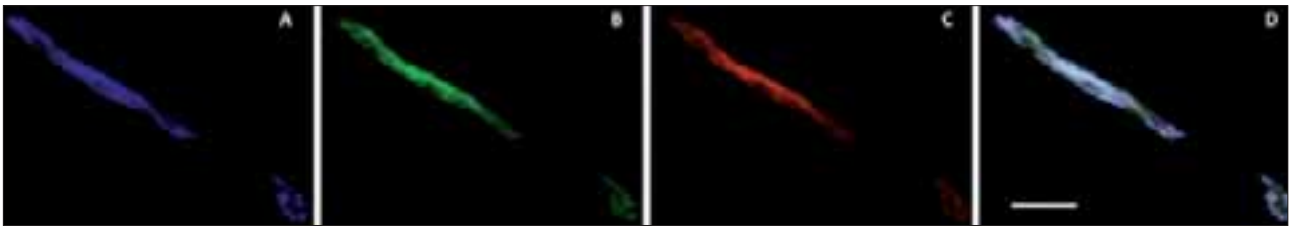

Figure 2. ***Representative photomicrographs of nerve fibers of guinea pig trachea, identified by the neurofilament antiserum (AMCA, blue fluorescence, A), showing immunoreactivities for PPAR-α (FITC, green fluorescence, B) and nNOS (TRITC, red fluorescence, C). Triple labeling (PPARα/nNOS/NF) shows colocalization of PPARα with nNOS within the nerve fibers (D) Original magnification for each image: x400. Scale bar: 100 μm***

process, but their role has not been well defined yet (3). Mast cells are thought to be involved in the pathogenesis of persulphate-induced asthma by releasing a number of inflammatory mediators. Direct irritation and damage of the airway mucosa is also thought to play a role (40,42). Either the immune system is involved or not, airway inflammation is the result of the action of any asthmogenic factor. Besides infiltrated inflammatory cells, various structural and resident cells all throughout the airways have also been shown to play a role in the pathophysiology of asthma: they include epithelial cells, smooth muscle cells, fibroblasts, alveolar macrophages, that could contribute to modulate airway inflammation (30). In each one of these cell types anti-inflammatory effects related to PPAR-α stimulation have been detected. Inflammatory processes are also known to cause significant damage to neurons and nerve fibers in many neurological disorders. Evidence has suggested that cellular peroxisomes may be important in protecting neurons from inflammatory damage, in which NO is known as a potent neurotoxic agent (33). In particular, fenofibrate (another well-known PPAR-α agonist) has been found to inhibit allergen-induced airway inflammation and to switch the immune response to allergen towards a Th1 profile (29). Little information is available about the effect of PPAR-α agonists on non-immunomediated airway inflammation (34).

The evidence that modulation of PPAR-α activity and peroxisomal function by fenofibrate attenuates NO-mediated neuronal and axonal damage has suggested a new therapeutic approach to protect against neurodegenerative changes associated with neuroinflammation (29,33,38,44).

Our results suggest that PPAR-α stimulation can be useful as well in protecting airway intrinsic inhibitory neurons against the functional impairment associated to inflammation. Our results are consistent with those of a recent study on the effect of gemfibrozil. The tracheal basal tone was assessed using isolated tracheal spiral strips of guinea pigs. Contractions were induced using acetylcholine, before and after inhibition of NOS Gemfibrozil produced relaxant effect on the basal tone in a dose-dependent manner and a dose-dependent reduction of the amplitude of acetylcholine induced tracheal contractions. Also, the inhibitory effect of this fibrate was completely abolished after inhibition of NOS. It completely inhibited serotonin-induced contractions as well, while having no effect on those caused by histamine or calcium gluconate (45).

We are aware that our data do not allow to discriminate between an indirect protective effect, due to the overall anti-inflammatory activity of the compound, and a direct effect, due to the stimulation of the PPAR-α receptors located in nerve fibers. Nevertheless, our data indicate that the effect of PPAR-α agonists on intrinsic nerves has to be carefully considered when evaluating the therapeutic potential of these compounds.

Our observations, taken together with the previous reports on the anti-inflammatory activity of PPAR-α agonists in the airways, suggest that these compounds may act as therapeutic agents for patients with asthma.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest, financial or otherwise, in this paper.

References

1) Tarlo S. M., Liss G. M., Dias C., et al. Assessment of the relationship between isocyanate exposure and occupational asthma. Am J Ind Med 1997; 32: 517-521.
2) Kopferschmitt-Kubler M. C., Ameille J., Popin E., et al. Occupational asthma in France: a 1-yr report of the observatoire National de Asthmes Professionnels project. Eur Respir J 2002; 19: 84-89.
3) Moscato G., Pignatti P., Yacoub M. R., et al. Occupational asthma and occupational rhinitis in hairdressers. Chest 2005; 128: 3590-3598.
4) Holgate S. T., Thomas M. Asthma. In Middleton's Allergy Essentials: First Edition. Elsevier Inc. 2017. https://doi.org/10.1016/B978-0-323-37579-5.00007-6
5) Chu, C., Artis, D., & Chiu, I. M. (2020). Neuro-immune Interactions in the Tissues. Immunity, 52(3), 464-474. https://doi.org/10.1016/j.immuni.2020.02.017
6) Lemière C., Vandenplas O. Occupational Allergy. Middleton's Allergy Essentials: First Edition. 2017; 361-375. https://doi.org/10.1016/B978-0-323-37579-5.00014-3
7) Dellabianca A., Faniglione M., De Angelis S., et al. Adenosine A1 and A3 receptor agonists inhibit nonadrenergic, noncholinergic relaxations in the guinea pig isolated trachea. Respiration 2009 78: 75-83.
8) Dellabianca A., Sacchi M., Anselmi L., et al. Role of carbon monoxide in electrically induced non-adrenergic, non-cholinergic relaxations in the guinea-pig isolated whole trachea. Br J Pharmacol 2007; 150: 220-6.
9) Barnes P. J. Neuroeffector mechanisms: the interface between inflammation and neuronal responses. J Allergy Clin Immunol 1996; 98: S73-S81.
10) Miura M., Ichinose M., Kimura K., et al. Dysfunction of nonadrenergic noncholinergic inhibitory system after antigen inhalation in actively sensitized cat airways. Am Rev Respir Dis 1992; 145: 70-74.
11) Belvisi M. G., Stretton C. D., Miura M., et al. Inhibitory NANC nerves in human tracheal smooth muscle: a quest for the neurotransmitter. J Appl Physiol 1992; 73: 2505-2510.
12) Jartti T. Asthma, asthma medication and autonomic nervous system dysfunction. Clinical Physiology 2001; 21: 260-269.
13) Mensing T., Marek W., Baur X. The influence of ammonium persulfate on guinea pig tracheal muscle tone: release of nitric oxide. Pharmacol Toxicol 1996; 78: 336-340.
14) Becker J., Delayre-Orthez C., Frossard N., et al. The peroxisome proliferator-activated receptor α agonist fenofibrate decreases airway reactivity to methacholine and increases endothelial nitric oxide synthase phosphorylation in mouse lung. Fundam Clin Pharmacol 2012; 26: 340-346.
15) Evans R. M. The steroid and thyroid hormone receptor superfamily. Science 1988; 240: 889-895.
16) Lamers C., Schubert-Zsilavecz M., Merk D. Therapeutic modulators of peroxisome proliferator-activated receptors (PPAR): a patent review (2008-present). Expert Opin Ther Pat 2012; 22: 803-841.
17) Belvisi M. G., Hele D. J., Birrell M. A. Peroxisome proliferator-activated receptor gamma agonists as therapy for chronic airway inflammation. Eur J Pharmacol 2006; 533: 101-109.

18) Spears M., Donnelly I., Jolly L., et al. Bronchodilatory effect of the PPAR-gamma agonist rosiglitazone in smokers with asthma. Clin Pharmacol Ther 2009; 86: 49-53.
19) Barnes P. J. (2012). Severe asthma: Advances in current management and future Therapy. J Allergy Clin Immunol 129: 48-59.
20) Donovan C., Bailey S. R., Tran J., et al. 2015 Rosiglitazone elicits in vitro relaxation in airways and precision cut lung slices from a mouse model of chronic allergic airways disease. Am J Physiol Lung Cell Mol Physiol 2015; 309: L1219-L1228.
21) Lee H. L., Rhee C. K., Kang J. Y., et al. Effect of intranasal rosiglitazone on airway inflammation and remodeling in a murine model of chronic asthma. Korean J Intern Med 2016; 31: 89-97.
22) Belvisi M. G., Mitchell J. A. Targeting PPAR receptors in the airway for the treatment of inflammatory lung disease. Br J Pharmacol 2009; 158: 994-1003.
23) Gervois P., Mansouri R. M. PPARα as a therapeutic target in inflammation-associated diseases. Expert Opin Ther Targets 2012; 16: 1113-1125.
24) Yoo S. H., Abdelmegeed M. A., Song B. J. Activation of PPARα by Wy-14643 ameliorates systemic lipopolysaccharide-induced acute lung injury. Biochem Biophys Res Commun 2013; 436: 366-371.
25) Hecker M., Behnk A., Morty R. E., et al. PPAR-α activation reduced LPS-induced inflammation in alveolar epithelial cells. Exp Lung Res 2015; 41: 393-403.
26) Gugliandolo E., Fusco R., Ginestra G., et al. Involvement of TLR4 and PPAR-α Receptors in Host Response and NLRP3 Inflammasome Activation, Against Pulmonary Infection With Pseudomonas Aeruginosa. SHOCK 2019; 51(2): 221-227.
27) Liu Y., Xie L., Yang M., et al. PPAR-α improves the recovery of lung function following acute respiratory distress syndrome by suppressing the level of TGF-β1. Mol Med Rep 2017; 16: 49-56.
28) Lakshmi S. P., Reddy A. T., Banno A., et al. PPAR Agonists for the Prevention and Treatment of Lung Cancer. Hindawi Publishing Corporation PPAR Research 2017; 8252796. doi:10.1155/2017/8252796.
29) Delayre-Orthez C., Becker J., Auwerx J., et al. 2008. Suppression of allergen-induced airway inflammation and immune response by the peroxisome proliferator-activated receptor-alpha agonist fenofibrate. Eur J Pharmacol 2017; 581: 177-184.
30) Becker J., Delayre-Orthez C., Frossard N., et al. Regulation of inflammation by PPARs: a future approach to treat lung inflammatory diseases? Fundam Clin Pharmacol 2006; 20: 429-447.
31) Dellabianca A., Faniglione M., De Angelis S., et al. Inhaled ammonium persulphate inhibits non-adrenergic, non-cholinergic relaxations in the guinea pig isolated trachea. Respiration 2010; 79: 411-419.
32) Cimini A., Benedetti E., Cristiano L., et al. Expression of peroxisome proliferator-activated receptors (PPARs) and retinoic acid receptors (RXRs) in rat cortical neurons. Neuroscience 2005; 130: 325-337.
33) Gray E., Ginty M., Kemp K., et al. Peroxisome proliferator-activated receptor-α agonists protect cortical neurons from inflammatory mediators and improve peroxisomal function. Eur J Neurosci 2011; 33: 1421-1432.
34) Enikeev O., Enikeeva S. A., Bikmetova N. R. PPAR-alpha agonist (fenofibrate) therapy in exacerbation control of chronic obstructive pulmonary disease of severe stage. Eur Respir J 2014; 44: P1514.
35) Adner M., Canning B.J., Meurs H., et al. Back to the future: re-establishing guinea pig in vivo asthma models. Clinical Science 2020; 134: 1219-1242.
36) Canning, B. J. Reflex regulation of airway smooth muscle tone. In Journal of Applied Physiology 2006; 101(3): 971-985.
37) Ricciardolo F.L.M., Nijkamp F., De Rose V., Folkerts G. The guinea pig as an animal model for asthma. Curr Drug Targets. 2008; 9(6): 452-65.
38) Wright J.L., Cosio M., Churg A. Animal models of chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol 2008; 295(1): L1-15.
39) Boyce J.A., Austen K.F. No audible wheezing: nuggets and conundrums from mouse asthma models. J Exp Med 2005; 201: 1869-1873
40) Mensing T., Marek W., Raulf-Heimsoth M., et al. Acute exposure to hair bleach causes airway hyperresponsiveness in a rabbit model. Eur Respir 1998; J 12: 1371-1374.
41) Curtis M. J., Bond R. A., Spina D., et al. Experimental design and analysis and their reporting: new guidance for publication in BJP. Br J Pharmacol 2015; 172: 3461-3471.
42) Munakata M., Huang I., Mitzner W., et al. Protective role of epithelium in the guinea pig airway. J Appl Physiol 1989; 66: 1547-1552.
43) Fidaleo M., Fanelli F., Ceru M. P., et al. Neuroprotective properties of peroxisome proliferator-activated receptor alpha (PPARα) and its lipid ligands. Curr Med Chem 2014; 21: 2803-2821.
44) Moran E. P., Ma J. X. Therapeutic Effects of PPARα on Neuronal Death and Microvascular Impairment. Hindawi Publishing Corporation PPAR Research 2015; 595426. doi:10.1155/2015/595426.
45) El Makkawy F. S., Ezzo A. M., Ouda E. A. E. A., et al. Pharmacological Effects of Gemfibrozil on Some Isolated Smooth Muscle Preparations Of Experimental Animals. The Egyptian Journal of Hospital Medicine 2017; 66: 1-17.

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