Etanercept attenuates short-term cigarette-smoke-exposure-induced pulmonary arterial remodelling in rats by suppressing the activation of TNF-α/NF-κB signal and the activities of MMP-2 and MMP-9

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The pathogenesis of cigarette-smoke-exposure-induced pulmonary vasculature impairment is unclear. Cigarette-smoke-exposure-induced the accumulation of tumour necrosis factor-alpha (TNF-α) and up-regulates the expression and activities of matrix metalloproteinases (MMPs) involved in smoke-induced vascular remodelling, which are important processes in the pathogenesis of vasculature impairment. The TNF-α antagonist Etanercept is an anti-inflammatory drug with a potential role in regulating MMP expression. To determine the effect of Etanercept on short-term smoke-induced pulmonary arteriole impairment and investigate its possible mechanism, male Sprague–Dawley rats were exposed to cigarette-smoke daily for two weeks in both the absence and presence of Etanercept. Cigarette-smoke-exposure-induced elevation of mean pulmonary artery pressures and medial hypertrophy of pulmonary arterioles were partially reduced by Etanercept. Up-regulation of the expression and activities of MMP-2 and MMP-9, induced by cigarette-smoke, were also suppressed significantly by Etanercept. Furthermore, Etanercept treatment significantly attenuated cigarette-smoke-induced TNF-α accumulation and activation of nuclear factor NF-κB signal. These results suggest that Etanercept have the protective effects in cigarette-smoke-induced pulmonary vascular remodelling, with the attenuation of the up-regulated expression and activities of MMP-2 and MMP-9 and activation of TNF-α/NF-κB signal pathway probably being involved as part of its mechanism. Our study might provide insight into the development of new interventions for vasculature impairment.

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1. Introduction

Environmental risk factors may play as a second hit on a genetic background in many pathogenic processes. One widespread environmental factor is cigarette-smoke-exposure, which is not only a risk factor for systemic vascular disease but also involves injury to the lung vasculature, which can lead to pulmonary arterial hypertension (PAH). A significantly increased percentage of smokers in populations with PAH, compared with the general population, indicates that tobacco-smoke-exposure could contribute to the development of PAH [30]. However, the mechanism is still unclear. Traditionally, elevated PAP and vascular remodelling were considered the result of loss of vascular bed, emphysematous lung destruction and hypoxic vasoconstriction. However, clinical–epidemiological studies show that mildly increased pulmonary artery pressure (PAP) often occurs in hypo-xaemic patients; whereas some patients display disproportionately elevated PAP and only very mild airflow obstruction [4]. These researches suggest that smoke-mediated pulmonary vasculature remodelling is a direct consequence after the up-regulation of vasoproliferative and vasoconstrictive mediators within the vascular walls [29,44].

In a rodent model studying the effects of cigarette-smoke, elevated PAP and vascular remodelling appeared before emphysema [19,34]. In addition, elevated PAP was induced by the dynamic alteration of pulmonary vasculature as an independent process, rather than being caused by hypoxia or emphysematous lung destruction [36,44]. Vascular cell proliferation and marked muscularisation of the arteries and arterioles adjacent to the alveolar ducts continues throughout the entire period of cigarette-smoke-exposure [38]. These structural changes, accompanied
by pulmonary artery remodelling, can be found in exposure to smoke over a period of two to three weeks [9]. Short-term exposure (two to three weeks) to cigarette-smoke invokes a proliferative response in both the airways and the lung vasculature [38,39]. The arterioles adjacent to the alveolar ducts have a greater response in both the endothelial cell and the vessel wall compartments compared with the muscular vessels situated adjacent to the airways [35]. This acute response appears to involve or to be invoked by oxidants and inflammatory factors [23]. Moreover, the initially proliferative response to exposure of cigarette-smoke becomes self-sustaining and is driven by mediators different from those in the later pathological process [15].

Vascular remodelling associated with elevated PAP, characterised by intimal fibrosis, medial hypertrophy and adventitial thickening, is associated with abnormal activation and/or deposition of the components of the extracellular matrix (ECM) in pulmonary vessels. Cowan et al. [11] showed that inhibitors of matrix metalloproteinases (MMPs) can prevent the progression of monocrotaline-induced PAH in rats. Although the exact mechanism involved in these pathogenic processes is disputed, MMP-mediated deposition of ECM seems to play an important role in this process, and ECM changes are also involved in the maintenance of the histological structure of the vessel wall and regulation of the contractility and proliferation of the pulmonary arterial smooth-muscle-cells [12,15,31]. Previous researches have shown that tumour necrosis factor-α (TNF-α) appears to be crucial in cigarette-smoke-induced inflammation and the development of vascular remodelling [7,42]. Moreover, TNF-α signal is also reported to regulate MMP activity in different cell types [18,20]. Wright et al. [42] reported that TNF-α is crucial to the increased production of MMPs in pulmonary arteries after cigarette-smoke-exposure, because TNF-α-receptor gene–knockout mice failed to show an up-regulation of MMP production.

Etanercept, a soluble TNF-α-receptor dimeric fusion protein, exerts its influence by suppressing TNF-α signal in rheumatoid arthritis and autoimmune diseases. In addition, Etanercept is efficacious in the inhibition of TNF-α and production of MMP-2 and MMP-9 in patients with idiopathic arthritis [2,33]. However, whether Etanercept has a therapeutic effect on cigarette-smoke-induced PAH has not yet been determined. Based on these findings, we hypothesise that Etanercept can prevent pulmonary vascular remodelling during cigarette-smoke-exposure through inhibition of the TNF-α signal pathway and suppression of up-regulation and activation of MMP expression. Therefore, the present study aims to investigate the effect of Etanercept in short-term cigarette-smoke-exposure-induced PAH rat model and the possible participation of MMPs in the mechanism.

2. Methods

2.1. Animals and experimental smoke-exposure

Male Sprague–Dawley rats, aged six to seven weeks and weighing 200–250 g, were supplied by the Shanghai SLAC Laboratory Animal Co., Ltd. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

The experimental groups were as follows, each group contained 6 animals:

1. Control groups (CTL) consisted of rats placed in ordinary room air.

2. Smoke-exposure-only groups (SM) were exposed to whole-cigarette-smokes (Great Harvest, China Tobacco Jiangsu Industrial Corp., Ltd, China; each cigarette contains 1 mg nicotine and 14 mg tar) in ventilated whole-body smoke chambers (70 × 50 × 50 cm) for 40 min each time, twice per day, six days/week for two weeks, as previously described [25]. The concentration of the total particulate matter in the smoke inside the exposure chambers was 200 ± 34 mg/m³, as determined by gravimetric analysis of the filters at the exhaust port during the duration of exposure.

3. Smoking-plus-Etanercept group (SMET) were treated with 0.4 mg/kg Etanercept (Enbrel®, Wyeth, USA) by subcutaneous injection three times a week during the smoke-exposure similar to the conditions in the SM group. Every injection was administered at least 6 h before smoke-exposure.

2.2. Haemodynamic analysis

Twenty-four hours after the previous smoke-exposure, rats were anaesthetised with urethane (1 g/kg, i.p.) and placed in a supine position, breathing room air. The mean PAP (mPAP) was measured as previously described [43] and recorded using a miniature liquid pressure transducer (RT14M2, 971004, Japan) equipped with a computerised data acquisition system (RM-6200C, China). A catheter (PE10, 427400, Becton Dickinson, USA) filled with heparin–saline solution (125 U/ml) was gently introduced through the right external jugular vein down to the right ventricle and pulmonary artery. All data were obtained from the steady-state wave forms recorded over a period of 5 min.

2.3. Morphology analysis

Assessment of pulmonary vascular morphology was carried out as previously described [43]. After the haemodynamic measurements, all animals were euthanised and the lungs were harvested. The left lung was dissected, snap-frozen in liquid nitrogen and stored at −80 °C until biochemical analysis. After fixation in 4% polyformaldehyde (pH 7.4) overnight, the right lung was embedded in paraffin and multiple 5 μm-thick sections were stained with (1) haematoxylin and eosin and Masson's trichrome stains. To assess the type of remodelling of the muscular pulmonary arteries concomitantly the respiratory bronchioles, at least 10 muscular arteries per section were examined, in a blinded fashion, using a computerised morphometric system (Quantimet 500; Leica, Cambridge, UK). The percentage medial wall thickness (MWT%) in fully muscularised arteries, with external diameters of 50–100 μm, was evaluated by calculating the MWT% along the shortest curvature, as follows:

\[ \text{MWT\%} = \left( \frac{\text{medical wall thickness} \times 2}{\text{external diameter}} \right) \times 100 \]

For the detection of MMP-2 and MMP-9 by immunohistochemistry (IHC), the sections were stained with mouse monoclonal anti-body to human MMP-2 (sc-10736, 1:100 dilution; Santa Cruz, USA), MMP-9 (sc-21733, 1:100 dilution; Santa Cruz, USA) using the UltraSensitive SP kit (Maixin, China). Preliminary experiments indicated that microwaving for 15 min in 0.01 M citric acid.

2.4. Real-time polymerase chain reaction

TNF-α, MMP-2 and MMP-9 mRNA transcripts were measured by Real-time quantitative reverse transcriptase polymerase chain reaction (Real-time-PCR). Total RNA was isolated from tissue samples using the TriPure® Isolation Reagent (Roche, Indianapolis, IN, USA). The reverse transcription was carried out with 300 ng of total RNA using the TaqMan reverse transcription reagent kit (Takala, Japan). Real-time-PCR was conducted using ABI PRISM® 7300 Real-time-PCR system using SYBR Green Real-time-PCR
Master Mix Reagent (Takala, Japan) in a single capillary tube, according to the manufacturer’s guidelines. The forward and reverse primers were each designed in a different exon of the target gene sequence, eliminating the possibility of amplifying genomic DNA. A positive result was determined by identifying the threshold cycle value at which the reporter dye emission appeared higher than the background. If the fluorescence signal was not detected within 40 cycles, the result was considered negative.

GADPH forward: 5'-CCATGGAGAAGGGCTGGG-3'  
GADPH reward: 5'-CAAAGTTGCTGATGACCC-3'  
TNF-α forward: 5'-GCCATTGCGCAGGAAGGC-3'  
TNF-α reward: 5'-CGCCACAGCTCTCTTG-3'  
MMP-2 forward: 5'-GATTTGGCAGTCAATACCT-3'  
MMP-2 reward: 5'-CAGAAGAATCTGAGCTTG-3'  
MMP-9 forward: 5'-CTTCGAGGCCACTCTACT-3'  
MMP-9 reward: 5'-CAGTGAAGTGGCTGAGT-3'

2.5. Preparation of homogenates and testing using enzyme-linked immunosorbent assay

The snap-frozen lungs were thawed, weighed, transferred to tubes on ice containing 1 ml of phosphate-buffered saline containing 0.1% Tween-20 (T-PBS) and Complete Mini Protease Inhibitor Cocktail tablets (Kaji, China), in a proportion of 1 tablet/10 ml of T-PBS stock reagent and then were homogenised at 4 °C. After centrifugation at 6000 × g for 15 min at 4 °C, the supernatants from the lung homogenates were transferred to clean microcentrifuge tubes, frozen on dry ice and thawed on ice. The total protein concentrations of the lung homogenates were determined using a bicinchoninic acid kit (Beyotime, China). The lung tissue homogenates were diluted with the T-PBS reagent to a final protein concentration of 500 μg/ml. The concentrations of TNF-α in the homogenate were determined using a rat enzyme-linked immunosorbent assay (ELISA) kit for TNF-α (R&D Systems, Minneapolis, MN, USA), according to the manufacturer’s protocols.

2.6. Gelatin zymography

To detect MMP-2 and MMP-9 activity, we conducted gelatin zymography, as previously described, with some modifications. Tissue lysates were prepared as described in detail in previous studies [18]. Sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE) was carried out using zymogram gelatin gels with 10% SDS–PAGE gels containing 0.1% gelatin (Sigma, USA); the gels were rinsed in double-distilled (dd)-H2O, followed by incubation with bulk volumes of renaturation buffer (2.7% Triton X-100 in dd-H2O) at room temperature for 1 h with gentle shaking. The enzyme activity was developed in 50 mM Tris (pH 7.5), 0.2 M NaCl, 5 mM CaCl2 and 0.2% Brij35 at 37 °C for 24 h. After incubation, the gel was stained by 0.5% Coomassie Blue R-250 (Bio-Rad Laboratories, Hercules, CA, USA) in 30% methanol and 5% acetic acid solution under continuous shaking for 2 h, then de-stained in 30% methanol and 5% acetic acid solution for 30 min, and then rinsed twice with de-staining solution to visualize the digested bands in the gelatin matrix. The gels were photographed and the averages of the band intensity were measured. Images were obtained and quantified with the Quantity One image-analysis software, v4.6.2. The data are presented as folds of the control.

2.7. Western blotting analysis

Total lysates and nuclear extracts of the cells were obtained. Equal amounts (50 μg) of protein were subjected to 10% SDS–PAGE and transferred onto a polyvinylidene fluoride membrane in a semi-dry system (Bio-Rad, USA). The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 and incubated with the specific antibodies directed against NF-κB (p65; sc-33020, 1:400 dilution; Santa Cruz, USA). The signals were developed using Super-Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA) and visualised using the Quantity One software, v4.6.2.

2.8. Statistical analysis

Statistical analysis was carried out using SPSS software (SPSS, v12.0, statistical package for Windows, Chicago, USA). Data are expressed as mean values (mean ± SD). The significance of the differences in the data between two groups was determined by the t-test. The mean values of the groups were compared using one-way ANOVA, followed by the Tukey’s honestly significant difference multiple-comparison procedure. A significant difference was accepted at p < 0.05.

3. Results

3.1. Etanercept attenuated the short-term smoke-exposure-induced increases in mPAP and pulmonary artery remodelling

To determine the effects of cigarette-smoke-exposure and Etanercept treatment on the pulmonary arteries of rats, we first examined the haemodynamics and histopathology of rat lungs. The baseline mPAP did not show any significant difference among the groups. As shown in Fig. 1D, cigarette-smoke-induced a significant increase in the mPAP (18.52 ± 3.46 mmHg) compared to the CTL group (11.76 ± 2.87 mmHg), whereas Etanercept administration partly attenuated the short-term smoke-exposure-induced increase in mPAP (13.98 ± 2.98 mmHg). However, no significant difference was found in the femoral arterial systolic blood pressure among the three groups (Data not shown). Compared to the CTL group, the number of small muscular pulmonary arteries concomitant with the respiratory bronchioles, pulmonary arterioles with intimal hyperplasia, hypertrophy of the vascular smooth-muscle and deposition of collagen around the vessel wall were much more significant in the lungs of the SM group rats (Fig. 1, A and B). In addition, the MWT% of the pulmonary arteries, which is a marker of pulmonary arterial remodelling, was significantly increased after cigarette-smoke-exposure (Fig. 1C). In contrast, treatment with Etanercept significantly reduced the smoke-induced increase in pulmonary arterial MWT% and elevated mPAP. These results confirmed that Etanercept treatment significantly attenuated acute smoke-exposure-induced increases in mPAP and pulmonary artery remodelling.

3.2. Etanercept reduced cigarette-smoke-exposure-induced up-regulation of MMP-2 and MMP-9 expression in pulmonary vasculature

To investigate whether changes in the expression and activity profiles of MMP-2 and MMP-9 are involved in cigarette-smoke-induced pulmonary artery remodelling and analyse the effects of Etanercept, IHC, Real-time-PCR and gel-zymography were used. The IHC results (Fig. 2, A–C) showed that MMP-2- and MMP-9-positive staining areas in the adventitia and hyperplastic intima of pulmonary arterioles in the smoke-exposed rat lungs were significantly increased compared to the CTL group. Consistently, cigarette-smoke-exposure-induced MMP-2 and MMP-9 mRNA expression was significantly up-regulated nearly three and two folds, respectively, in rat lungs compared to the CTL: this up-
5 regulation was significantly inhibited by Etanercept treatment (Fig. 2D, p < 0.05). Moreover, gel-zymography analysis indicated that cigarette-smoke-exposure-induced significantly higher MMP-2 and MMP-9 activities compared to CTL and that Etanercept treatment partially reversed the increased expression and activities of MMP-2 and MMP-9 induced by cigarette-smoke (Fig. 2E, p < 0.05).

3.3. Etanercept attenuated short-term smoke-exposure-induced activation of TNF-α/NF-κB signal in lungs

To determine whether TNF-α/NF-κB signal is involved in the therapeutic effects of Etanercept in cigarette-smoke-induced pulmonary vascular remodelling, the expression levels of TNF-α and NF-κB in rat lungs were assessed. Results showed that protein and mRNA expression of TNF-α in the lungs was significantly higher in the SM group than in the CLT group (Fig. 3, B and C). Etanercept treatment significantly decreased the TNF-α concentration in lung compared with the SM group. Nuclear and total NF-κB p65 were detected by Western blotting analysis (Fig. 3A) and the results showed that nuclear NF-κB p65 levels were markedly increased in the lungs of the SM group rats, compared to the lungs of the CLT group rats. Etanercept treatment resulted in a significant reduction of NF-κB p65 translocation compared to the SM group.

4. Discussion

The present study reported a novel finding regarding the therapeutic effect of Etanercept in cigarette-smoke-induced pulmonary artery remodelling. Our results showed that tobacco-smoke-
exposure over a period of two weeks induced intimal proliferation, muscularisation and collagen deposition in the small pulmonary arteries and arterioles adjacent to the alveolar ducts, leading to increased mPAP. Etanercept markedly reduced pulmonary artery remodelling and mPAP elevation caused by cigarette-smoke-exposure and partially reversed the smoke-induced up-regulation of expression and activity of MMP-2 and MMP-9 in rat lungs. Furthermore, Etanercept could inhibit smoke-induced TNF-α accumulation and NF-κB activation in rat lungs after a two-week exposure to tobacco-smoke; these interferences may contribute to the mechanism of Etanercept in smoke-induced pulmonary artery remodelling.

Studies on animals indicated that tobacco-smoke can directly induce pulmonary artery remodelling and pulmonary hypertension; moreover, this process cannot be explained by emphysematous destruction of the capillary bed [19]. Tobacco-smoke-induced vascular remodelling in guinea pig was found to be associated with notable levels of cellular proliferation and a sustained up-regulation of genes coding for mediators involved in vasoconstriction and proliferation [40,41], which lead to vascular
remodelling [41]. Some smokers develop severe chronic obstructive pulmonary disease (COPD), which may be associated with an increase in PAP. However, the severity of pulmonary hypertension in COPD is not correlated with the degree of airway obstruction [4]. A direct effect of tobacco-smoke on the pulmonary vasculature can thus be postulated and this hypothesis is supported by various studies [28,37], including our present work. Consistent with previous findings, in our work, we found increased mPAP and great muscularisation in the small pulmonary arteries and arterioles adjacent to the alveolar ducts after cigarette-smoke-exposure for two weeks.

Clinical studies have shown that fragmentation of the internal elastic lamina and an increase in the activities of MMPs occur in the pulmonary arteries of PAH patients [21]. Higher mRNA expressions and enzymatic activities of both MMP-2 and MMP-9 were also found in the lungs of PAH animals [24,42]. Cigarette-smoke-exposure is believed to cause endothelial cell injury in pulmonary arteries and inflammatory response in lungs [41], in addition to inducing the secretion of MMPs from injured endothelial and inflammatory cells, such as mast cells and macrophages [7,42]. Among the MMPs, MMP-2 and MMP-9 degrade collagen more efficiently than the others; in addition, they have been implicated in the pathogenesis of vascular remodelling and are believed to be therapeutic targets in vascular diseases [13,17,31]. Our results in the present study show markedly increased expression and activities of MMP-2 and MMP-9 in rat lungs after two weeks of cigarette-smoke-exposure, which suggests that cigarette-smoke-induced ECM remodelling of pulmonary arteries and an inflammatory response in the lung tissue. A significant correlation exists between the right ventricular function and serum TNF-α level in PAH patients [32]. In vivo research has shown that TNF-α-receptor knockout significantly inhibits smoke-induced PAH in mice [8]. Our study also found marked increases of TNF-α expression in rat lungs after two weeks of cigarette-smoke-exposure, which implies that TNF-α signal plays an important role in cigarette-smoke-induced vascular remodelling. Although the exact mechanism of this process is unclear, recent reports indicate that TNF-α is involved in the process by its induction of vascular smooth-muscle-cell proliferation, expression of pro-inflammatory cytokines and production of MMPs [3,6,35].

Etanercept, a biological agent used in the clinical treatment of rheumatoid arthritis, is a fusion protein consisting of two identical chains of the recombinant extracellular TNF receptor II monomer fused with the Fc domain of human IgG. However, nothing is known about its potential role in pulmonary vascular diseases. Because TNF-α plays a central role in the inflammatory process in PAH and Etanercept binds both TNF-α and lymphotoxin and inhibits their activity, we hypothesise that Etanercept may prevent pulmonary vascular remodelling in PAH resulting from cigarette-smoke-exposure. Our study shows, for the first time, that Etanercept treatment attenuates the increased mPAP induced by cigarette-smoke-exposure, in addition to suppressing the thickening of small pulmonary arteries and the muscularisation of peripheral pulmonary arterioles induced by short-term cigarette-smoke-exposure. Furthermore, our results indicate that Etanercept reduces collagen deposition and destruction in pulmonary arteries, induced by cigarette-smoke, in association with an obvious suppression of MMP-2 and MMP-9 expression and activities. Moreover, a considerable interaction exists between the MMPs, with MMP-2 activating pro-MMP-9; these interactions may occur in the pulmonary vessels also and help to explain the therapeutic mechanism of Etanercept. An interesting finding is that Etanercept...
remarkably reduced cigarette-smoke-induced pulmonary inflammation and this inhibition was accompanied by a significantly decreased expression of the inflammatory cytokine TNF-α. Some studies have previously reported that TNF-α is closely linked to inflammatory responses and the development and maintenance of arterial remodelling through the release of cytokines [7,42]. TNF-α-mediated induction of the expression of the MMP-2 and MMP-9 genes is well described and could be responsible for the overexpression and activity of these molecules, as found in this research. TNF-α induces MMP-2 and MMP-9 activity markedly and increases their mRNA levels through its action on the promoter regions of the MMP-2 and MMP-9 genes [22]. On the contrary, the general action of MMPs in physiological and pathological inflammatory processes is through the regulation of the activity of inflammatory cytokines [1]. MMPs can mediate the proteolytic process that leads to the release of the soluble, active form of TNF-α from a cell membrane-anchored inactive form [10,16]. Therefore, there is a complex interaction between the MMPs 2 and 9 and the inflammatory cytokine TNF-α, which might be involved in the protection mechanism of Etanercept against cigarette-smoke-induced PAH. Our data suggests that the inhibitory effect of anti-TNF-α therapy may thus offer a twofold efficacy through (1) the reduction in the levels and activities of MMP-2 and MMP-9 and (2) the inhibition of the processing of the TNF-α precursor into its active molecular form.

The inflammatory responses following exposure to cytokines such as TNF-α are greatly dependent on the activation of NF-κB [20,42]. The sequestration of NF-κB is regulated by the translocation of the heterodimers of p65. Our results showed that cigarette-smoke-exposure up-regulated NF-κB p65 expression in the nucleus and that Etanercept treatment suppressed NF-κB p65 translocation. The transcription factor NF-κB is required for the full induction of both MMP-2 and MMP-9 by TNF-α [14,18]. Competition with the p65 subunit of NF-κB for binding to the MMP promoter suppressed the expression of MMPs [27]. Inhibition of MMPs is associated with the transcriptional activity of NF-κB [5]; in addition, TNF-α is known to be regulated by NF-κB activity [26]. Considering all these results together, the effects of Etanercept in short-term cigarette-smoke-exposure-induced PAH can be proposed to be mediated by the down-regulation of NF-κB p65 translocation, thus regulating the activities of TNF-α and the MMPs. However, our data do not identify a clear effect of Etanercept in relation to the inflammatory cascade observed in cigarette-smoke-induced pulmonary vascular remodelling. The experimental period of 14 days used in our study is relatively short and cannot provide additional information on the long-term outcome of smoke-induced pulmonary vascular remodelling following this modified-treatment approach.

5. Conclusion

In summary, our data showed that Etanercept effectively attenuated not only pulmonary arterial remodelling and mPAP elevation, but also the up-regulated expression and activities of MMP-2 and MMP-9 induced by short-term cigarette-smoke-exposure. Moreover, activation of the TNF-α/NF-κB signal pathway may serve as part of its mechanism. These findings provide a new insight into the role of Etanercept in cigarette-smoke-induced pulmonary vascular remodelling.

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that could inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "Etanercept attenuates short-term cigarette smoke-exposure-induced pulmonary arterial remodelling in rats by suppressing the activation of TNF-α/NF-κB signal and the activities of MMP-2 and MMP-9."

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Appendix A. Supplementary material


References


