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Cytokine-mediated xanthine oxidase upregulation in chronic obstructive pulmonary disease's airways

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Abstract

Reactive oxygen species have been reported to be involved in the airway inflammatory process of chronic obstructive pulmonary disease (COPD). The aim of this study was to quantify the activity of xanthine oxidase (XO), which generates a potent radical superoxide anion in COPD airways.

Thirteen stable COPD patients and 10 healthy subjects participated in this study. We collected the epithelial lining fluid using a newly developed microsampling technique, and quantified of cytokines responsible for the XO gene upregulation.

The XO activity was significantly increased in COPD patients compared with that in healthy subjects. A significant negative correlation was found between the XO activity and the $\%FEV_1$ values. The level of tumor necrosis factor-α, interleukin-1β, and interferon-γ in COPD patients was significantly higher than that in healthy subjects. Both the amount of tumor necrosis factor-α and interleukin-1β were significantly correlated with the degree of XO activity.

These results suggest that the XO activity is increased in COPD airways, possibly due to its gene upregulation by proinflammatory cytokines. Because the XO activity was significantly correlated with the degree of airway obstruction, these cytokine–XO production pathways may play a key role in the inflammation of COPD.

Keywords: Oxidative stress; Superoxide; TNF-α; IL-1β; IFN-γ

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a condition characterized by airway inflammation and airflow limitation that is progressive and largely irreversible [1–3]. Although COPD is a major cause of morbidity and mortality in the world [4], the pathogenesis of this disease has not yet been fully elucidated.

There is increasing evidence that an oxidant/antioxidant imbalance occurs in COPD [5–7]. Oxidants including superoxide anion (O_2^-), hydrogen peroxide and peroxynitrite cause airway inflammation by means of tissue injury [5], the activation of matrix metalloproteinases [8], the inactivation of α_1-antitrypsin [9], and enhanced production of the potent neutrophil chemoattractant interleukin (IL)-8 [10]. Therefore, these oxidants appear to be involved in the pathophysiology of the airway inflammation in COPD patients. Recently, endogenously generated oxidants have been thought to be important in COPD [5,6]. Among these, O_2^- is the most important molecule since other oxidants are derived from this molecule.

Xanthine oxidoreductase (XOR) is a rate-limiting enzyme of purine catabolism that exists in two forms, as xanthine dehydrogenase (XD) and as xanthine oxidase (XO) [11,12]. XOR, particularly in the XO form, generates reactive oxygen species such as O_2^-, hydroxyl radicals and hydrogen peroxide. It has been reported that XO was enhanced in an animal model of asthma [13] and in virus-induced pneumonia in mice [14]. These studies showed that O_2^- mediated by XO caused both airway and lung parenchymal inflammation. Previously, we have reported that the XO activity in sputum from patients with COPD

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was increased compared with that of healthy subjects [15]. Similarly, Pinamonti et al. [16] have reported that the XO activity was increased in bronchoalveolar lavage fluid from COPD patients. However, these methodologies using sputum and bronchoalveolar fluid samples are semiquantative.

Further, the mechanisms responsible for the upregulation of the XO activity are still unclear. Some proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1β, and interferon (IFN)-γ have been reported to upregulate XO gene expression in bovine renal epithelial cells [17], rat alveolar macrophages [18], and in human mammary epithelial cells [19]. In a rat model, IL-1 and IFN-γ intratracheal instillation cause enhanced XO activity in the lungs [20]. Therefore, it is possible that these proinflammatory cytokines are excessively produced in COPD airways and upregulated the XO gene expression.

The aim of this study was to quantify the XO activity in COPD airway epithelial lining fluid (ELF) using a new bronchoscopic microsampling technique [21,22]. We also quantified proinflammatory cytokines in the ELF that are responsible for XO gene upregulation.

2. Methods

2.1. Subjects

Thirteen stable Japanese COPD patients and 10 Japanese healthy subjects participated in the present study. Forced expiratory volume in 1 s (FEV_1) was assessed with a dry rolling seal spirometer (Chestac 11, Chest Co., Tokyo, Japan). Table 1 shows the characteristics of the study subjects. None of the healthy subjects were atopic nor had abnormal lung function. They did not have clinical manifestations of bronchial asthma such as recurrent episodes of wheezing. COPD was diagnosed according to the criteria of Global Initiative for Chronic Obstructive Lung Disease [1]. The lungs of all COPD patients showed low-attenuation areas in computed tomographic studies. All subjects in both groups had quit smoking at least 1 year before the study. No subject had had a respiratory tract infection during the 1-month preceding the study, or systemic or inhaled steroid therapy during the 2 months prior to the study. Approval by the Tohoku University Ethics Committee for Clinical Investigations and informed consent were obtained.

Table 1
Characteristics of study subjects

	Healthy subjects	COPD patients
Age (years)	65.8±5.0	65.8±2.9
Sex (M:F)	6:4	10:3
FVC (l)	3.08±0.38	2.89±0.25
FEV_1 (l)	2.49±0.27	1.52±0.21*
FEV_1/FVC (%)	81.9±1.4	50.6±3.7*
%FEV_1 (%)	106.7±13.2	67.7±7.0*
Pack-years	–	53.0±9.6*

COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; %FEV_1, percent predicted FEV_1. Data are given as mean±SEM. *$p<0.01$ compared with healthy subjects.

2.2. Bronchoscopic microsampling

ELF was obtained using a bronchoscopic microsampling technique according to the method of Ishizaka and co-workers [21,22]. After all subjects were intramuscularly administered with 25 mg of hydroxyzine and 0.5 mg of atropine, local anesthesia of the upper respiratory tract was achieved with 5 ml of 4% lidocaine. A flexible fiberoptic bronchoscope (P250, Olympus, Tokyo, Japan) was inserted into the right bronchus intermedius. After the channel of the bronchoscope was flushed with air, the microsampling probe (BC-401C, Olympus, Tokyo, Japan) was inserted through the channel of the bronchoscope. The probe consists of a 1.8 mm outer diameter polyethylene sheath and a 1.1 mm inner polyester fiber rod probe attached to a stainless steel guide wire. Next, the inner probe was advanced into the bronchial lumen slowly to avoid injuring the bronchial wall. The inner probe was gently contacted with the bronchial wall for 15 s. The inner probe was then withdrawn into the outer sheath, and both devices were withdrawn simultaneously through the channel. We could obtain 18.0±0.9 μl ELF per probe. The same procedure was repeated at the same site three times. The wet inner probe was sectioned at 3 cm from its tip and placed in a tube. One milliliter saline was added to the tube to elute ELF and the tube was vortexed for 1 min. The solution contained 144±14 μg/ml protein, and was stored at −80 °C until use for the assay.

2.3. Measurement of XO enzyme activity in the ELF

XO activity was measured according to previous studies [14,15]. Briefly, an inhibitor cocktail (ice-cold 50 mM potassium phosphate buffer containing 2 mM ethylenediaminetetraacetic acid, 2 mM *p*-amidinophenyl methanesulfonyl fluoride hydrochloride, 10 mM DL-dithiothreitol and 0.5 μg/ml leupeptin hydrochloride) was added to the obtained samples. Next, the samples were centrifuged at 790 g for 10 min at 4 °C and the supernatants were re-centrifuged at 100,000 g for 1 h at 4 °C. The supernatants were filtered with a 0.45 μm filter unit (SLHV013OS, Millipore, Bedford, MA, USA). In order to remove the endogenous substrate of XO (e.g. xanthine and hypoxanthine), the supernatants were dialyzed for 5 h against 5 l of 50 mM PBS (pH 7.4) at 4 °C with cellulose tubing (Seamless Cellulose Tubing, size 8/32; Sankou Pure Chemical Industries, Tokyo, Japan). Pterin was added to each dialyzed sample as a substrate for XO, and the assay mixture, which contained 9 μM pterin, was prepared. Reactions were allowed to proceed for 1 h at 37 °C.

All samples were assayed for their XO activity using a spectrofluorometer (model 650–40; Hitachi Ltd, Tokyo, Japan) with excitation at 345 nm and emission at 390 nm. To confirm the specificity of the activity, 20 μM allopurinol was added to the sample. The activity was expressed as the formation of isoxanthopterin, and corrected by the protein concentration of the sample.

2.4. Measurement of proinflammatory cytokine amounts in the ELF

Using the ELF sample processed as above mentioned (Section 2.2), the levels of TNF-α, IL-1β and IFN-γ in the ELF were measured by ELISA (Quantikine, R&D systems, Minneapolis, MN, USA). The detection threshold of TNF-α, IL-1β and IFN-γ were 1.6, 1.0, and 8.0 pg/ml, respectively. These cytokine levels were corrected by the protein concentration of each sample.

2.5. Drugs

Pterin, isoxanthopterin, ethylenediaminetetraacetic acid disodium salt dihydrate, leupeptin hydrochloride, dithiothreitol and allopurinol was purchased from Sigma Chemical Co. (St Louis, MO, USA). *p*-Amidinophenyl methanesulfonyl fluoride hydrochloride were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan)

2.6. Statistical analysis

All data were presented as mean ± SEM. To determine significant difference, the Mann–Whitney *U*-test was performed. Pearson's correlation analysis was used to assess the correlation between the XO activity values and cytokine levels, and between the XO activity values and the percent predicted FEV_1 (%FEV_1) values. Probability values of less than 0.05 were considered significant.

3. Results

Table 1 shows the characteristics of the subjects who participated in the present study. In the present study, the collection of ELF samples with the microsampling probe was accomplished without serious adverse events such as pneumonia or pulmonary hemorrhage.

The XO activity in the ELF is shown in Fig. 1. The XO activity in the patients with COPD was significantly increased compared with healthy subjects (114.1 ± 16.2 vs 31.4 ± 7.7 nmol isoxanthopterin/mg protein h^{-1}, $p<0.01$). Furthermore, there was a significant negative correlation between the values of the XO activity and the %FEV_1 values in all subjects ($r=-0.61$, $p<0.01$; Fig. 2).

Fig. 3 shows the amount of proinflammatory cytokines in the ELF. The levels of TNF-α in the patients with COPD were significantly higher than those in the healthy subjects

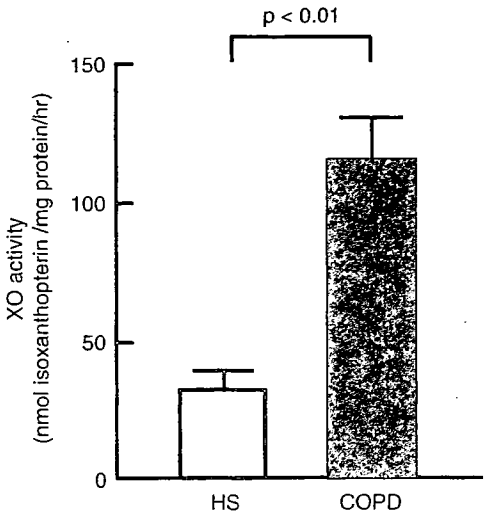

Fig. 1. Xanthine oxidase (XO) activity in the epithelial lining fluid from healthy subjects (HS, open bar) and COPD patients (COPD, closed bar).

(105.5 ± 11.1 vs 60.6 ± 3.3 pg/mg protein, $p<0.05$; Fig. 3A). We found similar results in the levels of IL-1β (32.1 ± 4.8 vs 16.1 ± 2.4 pg/mg protein, $p<0.05$; Fig. 3B) and IFN-γ (92.1 ± 9.0 vs 56.5 ± 8.3 pg/mg protein, $p<0.05$; Fig. 3C).

As shown in Fig. 4, each amount of TNF-α ($r=0.66$, $p<0.01$; Fig. 4A) or IL-1β ($r=0.68$, $p<0.01$; Fig. 4B) was significantly correlated with the values of XO activity in ELF. However, there was no significant correlation between the amount of IFN-γ and the XO activity values in the ELF (Fig. 4C).

4. Discussion

We have shown that the XO activity in the ELF of COPD patients was significantly higher than that in healthy subjects. In addition, the values of the XO activity were significantly correlated with the values of %FEV_1, suggesting that this enzyme may be involved in the inflammatory process of COPD.

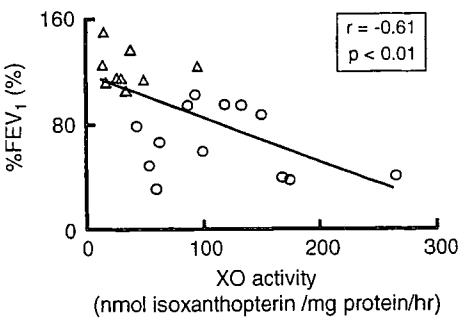

Fig. 2. Relationship between xanthine oxidase (XO) activity values and % predicted FEV_1 values. The straight line and *p* value correspond to the fitted regression equation of Pearson's correlation analysis. Open triangles, healthy subjects; open circles, COPD patients.

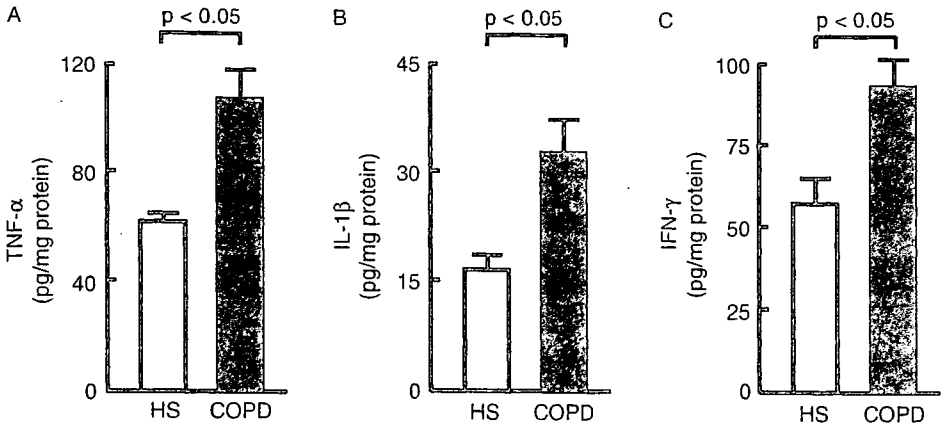

Fig. 3. Levels of TNF-α (A), IL-1β (B) and IFN-γ (C) in the epithelial lining fluid from healthy subjects (HS, open bar) and COPD patients (COPD, closed bar).

XOR including XO and XD is a rate-limiting enzyme of purine catabolism that catalyzes the oxidative hydroxylation of hypoxanthine to xanthine and xanthine to uric acid [11,12]. In particular, the XO form generates reactive oxygen species such as O_2^-, hydroxyl radicals and hydrogen peroxide. Although XD is the primary gene product of XOR, it can be converted reversibly or irreversibly into XO by oxidation or by proteolytic cleavage. The upregulation of XO enzyme activity has been shown under various conditions including a bronchial asthma model [13] and viral pneumonia [14]. Recent studies have shown that the XO activity in the airways of COPD patients is significantly higher than that in healthy subjects semiquantatively using induced sputum [15] or bronchoalveolar lavage fluid samples [16]. In the present study, we first quantified the XO activity in the ELF. The XO activity of the COPD patients was 3.6 times higher than that of the healthy subjects.

The bronchoscopic microsampling technique employed in the present study has methodological advantages compared with sputum or BALF sampling techniques by enabling quantitative analysis. Also, we could accomplish this more safely than when collecting BALF, particular in patients with pulmonary diseases. These advantages encouraged us to utilize this technique to obtain samples and to assess the oxidative burden quantitatively in COPD airways.

In the present study, we found that there was a significant negative correlation between the values of XO activity and $\%FEV_1$ values as shown in Fig. 2. O_2^- not only causes tissue injury directly, but also enhances the production of chemokines [10]. Furthermore, the activation of matrix metalloproteinases [8] and the inactivation of antiprotease [9] by O_2^- lead to an imbalance in protease/antiprotease, destruction of the lung parenchyma, and a decrease in the lung elastic recoil. Through these mechanisms, O_2^- derived from XO may contribute to the airway obstructive changes in patients with COPD. Therefore, upregulation of XOR activity may be an important factor for the inflammatory process of COPD airways.

In the present study, we have also demonstrated that the airway levels of proinflammatory cytokines such as TNF-α, IL-1β and IFN-γ were significantly increased in the COPD subjects compared with those of the healthy subjects. Among these three cytokines, the levels of TNF-α and IL-1β in the ELF were significantly correlated with the values of XO activity, indicating that these two proinflammatory

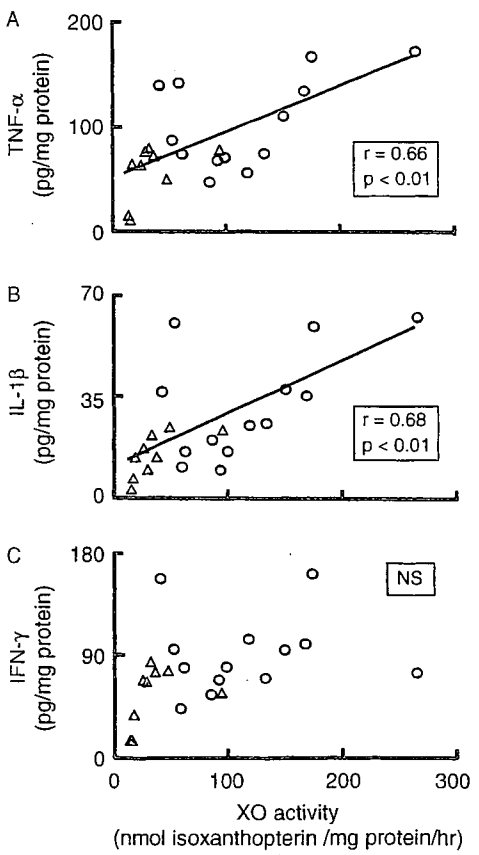

Fig. 4. Relationship between xanthine oxidase (XO) activity values and levels of TNF-α (A), IL-1β (B) and IFN-γ (C). The straight line and *p* value correspond to the fitted regression equation of Pearson's correlation analysis. NS, not significant; open triangles, healthy subjects; open circles, COPD patients.

cytokines may be important for the gene upregulation of XO.

It has been reported that the TNF-α level was increased in induced sputum in COPD patients [23]. In addition, TNF-α was reported to induce the conversion of XD to XO in rat pulmonary artery endothelial cells [24]. The levels of IL-1β in BALF from current smokers have been thought to be higher than in those who have never smoked [25]. It has been reported that IFN-γ mRNA expression was increased in patients with chronic bronchitis compared with healthy subjects [26]. In the present study, we showed that the protein level of IFN-γ was increased in COPD patients compared with healthy subjects. Further, we are the first to demonstrate the relationship between these proinflammatory cytokines and the XO activity in COPD airways.

The XO activity is generally regulated by its gene expression. Previous studies showed that the XOR gene expression was markedly stimulated by the proinflammatory cytokines TNF-α, IL-1β, and IFN-γ in bovine renal epithelial cells [17], rat alveolar macrophages [18] and human mammary epithelial cells [19]. Moreover, in a rat model, IL-1 and IFN-γ intratracheal instillation caused an enhancement of the XO activity in the lungs [20]. Taken together, these cytokines seem to be responsible for the XO gene upregulation in COPD airways. Significant positive correlation between the levels of TNF-α or IL-1β and the values of XO activity is supporting the hypothesis.

The localization of XOR in human lung is barely understood. In immunohistochemical studies, no XOR activity was demonstrated in human lung tissues [27]. But we and other researchers detected XOR activity in the samples from human lung (e.g. sputum, BALF, or ELF). Rouquette and co-workers reported that XOR was not only distributed in cytoplasm, but also on the cell surface of three cell types [28]. Although we did not verify the XOR localization in human lung, XOR was presumably secreted from lung components such as bronchial epithelial cells, pulmonary epithelial cells or alveolar macrophages.

In conclusion, we have first shown quantitatively that the XO activity is upregulated in COPD airway lining fluid using a new bronchoscopic microsampling technique. We have also found evidence that the proinflammatory cytokines including TNF-α and IL-1β may be responsible for the XO gene upregulation. Because the XO activity was significantly correlated with the degree of airway obstruction, these cytokine-XO production pathways may play a key role in the inflammation and airflow limitation of COPD.

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Microvascular hyperpermeability in COPD airways

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Microvascular hyperpermeability in COPD airways

Chronic obstructive pulmonary disease (COPD) is characterised by an abnormal inflammatory response of the lungs. An increase in the albumin concentration in the sputum of COPD patients has previously been reported.[1] This may suggest that the airway microvascular permeability is increased in COPD airways because the albumin comes from the vasculature via endothelial contraction at post-capillary venule lesions. However, measurement of sputum samples has some limitations such as contamination by saliva. We have measured the albumin concentration of the airway lumen in patients with COPD using a new direct technique for collecting airway epithelial lining fluid.[2]

Eighteen untreated patients with peripheral type lung cancer undergoing a bronchoscopic examination for the diagnosis were recruited to the study. Approval was obtained from the Wakayama Medical University ethics committee and the patients gave their written informed consent. The mean (SE) age of the patients was 70.4 (2.0) years. Eight patients were current smokers, seven ex-smokers, and three non-smokers. Five of the subjects did not have COPD, four were at risk (stage 0), six had moderate COPD (stage II), and three had severe COPD (stage III) according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of the severity of COPD.[3] Epithelial lining fluid was collected using a microsampling probe under bronchoscopy at the main or intermediate bronchus on the tumour absent side. The albumin concentration in the extracted ELF was measured and normalised by the values in the serum.

The normalised airway albumin values showed a strong correlation with the forced expiratory volume in 1 second % predicted (%FEV_1) values ($r = -0.727$, $p = 0.0006$; fig 1). There was no significant difference in the airway albumin values according to smoking status (non-smokers: mean (SE) 1.21 (0.29)%, ex-smokers: 1.23 (0.28)%, current smokers: 1.14 (0.28)%) or age. These data suggest that an increase in airway microvascular permeability may be involved in the inflammatory and subsequent obstructive process of COPD.

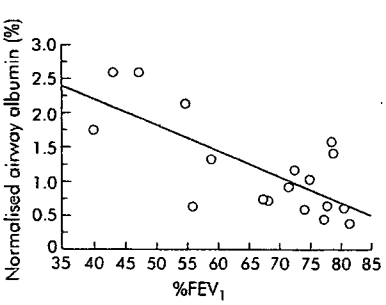

Figure 1 Relationship between normalised airway albumin and forced expiratory volume in 1 second % predicted (%FEV_1). Normalised airway albumin values were calculated as values of epithelial lining fluid/values of serum.

The precise mechanism of the microvascular hyperpermeability observed in COPD has not been well characterised. We have recently reported that oxidative and nitrosative stress is exaggerated in COPD airways.[4 5] Reactive oxygen/nitrogen species such as superoxide anion and peroxynitrite may participate in the microvascular hyperpermeability of COPD airways.

At present some airway/pulmonary cells (including epithelial cells, neutrophils, and macrophages) are considered therapeutic targets for future COPD treatment. In addition to these cells, the airway microvasculature may also be a target in the treatment of COPD. Furthermore, airway albumin values may be a good marker for the efficacy of COPD treatment.

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Assessing the validity of genetic association studies

We read with interest your approved guidance on the key issues which should be considered in preparing a genetic association study to be acceptable for publication in *Thorax*.[1 2] While we agree with several points in this guidance, other points we consider to be exaggerated or, at best, controversial. We note that, in the eight genetic association studies published in *Thorax* since 2004, some of them do not conform to this guidance with regard to population size, number of polymorphisms studied, and their functionality. This is seen clearly in the latest published association study by Yarden and colleagues[3] who examined four polymorphisms in the *TNFα* gene in patients with cystic fibrosis. Three of the studied polymorphisms were without functional information; no assessment of linkage disequilibrium, haplotype analysis or correction for multiple comparisons had been performed; and the population size—even after pooling the two different ethnic groups—showed that the study was underpowered.

With regard to the population size required in your guidance, the numbers in table 1 are too high (regardless of the typing error that caused the cases required for minor allele frequencies of 0.2 and 0.4 to be reversed). The reason for this is the unusual use of 90% power instead of the widely applied 80%. In fact, 80% power is the default for the online genetic power calculator you yourself provided in your editorial. Using this default of 80%, much smaller numbers of cases could be obtained and considered as having enough power. For example, with the relative risk set at 2, only 130 or 170 cases are required when the "minor allele frequency" is 0.4 and 0.2, respectively. We therefore think that your assumption that a study of 150 asthmatics and 150 controls is unlikely to be adequately powered needs some modification (such as adding to it if the minor allele frequency is less than 0.3).

As far as the functionality of a polymorphism is concerned, we agree that studying known functional polymorphisms rather than random polymorphisms in the gene of interest is advantageous in terms of detecting true disease associated variants. However, restricting genetic association studies to functional polymorphisms may lead to important polymorphisms being missed because the functional effects of many polymorphisms are difficult to assess, either as a result of technical problems (such as intronic, coding synonymous, or polymorphisms that are far upstream or downstream from the studied gene) or because of an absence of the full knowledge of the gene function and how it might be influenced by the polymorphism.

With regard to population stratification, there is no doubt that a study population that contains ethnically or geographically unmatched subjects may lead to spurious results, and we do not think any researcher would undertake an association study based on such a population. However, your assumption that even an apparently homogenous population may show substratification and your request that study populations should

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Clinical Efficacy and Safety of Transdermal Tulobuterol in the Treatment of Stable COPD: An Open-Label Comparison with Inhaled Salmeterol

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Abstract

Background: Long-acting bronchodilators are recommended for the management of stable COPD to relieve symptoms and improve quality of life. The tulobuterol patch (Hokunalin®) is a transdermal patch preparation of the β_2-adrenoceptor agonist (β_2-agonist) tulobuterol designed to yield sustained β_2-agonistic effects for 24 hours when applied once daily.

Objective: To compare the effectiveness of tulobuterol patch and inhaled salmeterol (Serevent® Diskus) in the treatment of stable COPD.

Study design: Clinically stable COPD patients (age ≥40 years, postbronchodilator FEV_1/FVC <70%, and postbronchodilator FEV_1 <80% predicted) were enrolled in a multicenter, open-label randomized study. After a 2-week run-in period, patients were administered either tulobuterol (2mg once-daily applied as a patch) or salmeterol (50μg per inhalation, twice a day) for 12 weeks.

Results: Data for 92 patients (46 each for each treatment group) were analyzed. There were no significant differences in baseline characteristics in the tulobuterol versus salmeterol groups: age, 69.2 ± 7.4 vs 71.6 ± 7.3 years; male, 91% versus 96%; and patients with stage II (III) COPD, 32.6% (67.4%) versus 50% (50%). FEV_1, FVC, and PEF improved during treatment in both groups compared with baseline, with no significant between group differences. The total St George's Respiratory Questionnaire (SGRQ) score was significantly improved relative to baseline in the tulobuterol group at 8 weeks (−4.7 units [U]), but not in the salmeterol group at all timepoints. Domain analysis of the SGRQ scores revealed significant improvement in the symptom score relative to baseline in the tulobuterol group at weeks 4 (−6.9U), 8 (−12.0U), and 12 (−11.7U), but not in the salmeterol group in any of the domains tested. Medical Research Council dyspnea scale score improved during treatment in both groups, with no significant differences between groups. Compliance with the treatment regimen was significantly better in the tulobuterol than in the salmeterol group (98.5% vs 94.1%; $p < 0.05$).

Conclusion: These findings indicate that once-daily transdermal sustained-release tulobuterol is as effective or better than the inhaled long-acting β_2-agonist salmeterol in the management of stable COPD, with significant effects on quality of life.

COPD is an inflammatory lung disease caused by inhalation of toxic particles such as tobacco smoke. The number of patients with COPD is increasing worldwide, as a large percentage of the population approaches old age. GOLD (Global Initiative for Chronic Obstructive Lung Disease) recommends that COPD be treated and managed according to severity of airflow limitation, and considers bronchodilators to play important roles in the pharmacotherapy of stable COPD.[1] The majority of guidelines for the management of COPD, including those issued by GOLD[1] and many regional or local academic societies,[2,3] recommend the use of long-acting, inhaled rather than oral bronchodilators, because of the lower incidence and severity of adverse drug reactions, and sustained bronchodilation.[1-3] Although inhaled bronchodilators, which can exert direct effects on affected areas, are the most preferable drug form in terms of efficacy and safety, elderly patients with COPD, who often suffer from concomitant diseases, may not inhale sufficient amounts of the drug, and COPD patients with severe respiratory dysfunction or acute exacerbation may have particular difficulty with drug inhalation. Alternative drugs with excellent efficacy and safety are therefore needed.

A formulation of tulobuterol using a sustained transdermal delivery system and containing 2mg of the β_2-adrenoceptor agonist (β_2-agonist) in a patch preparation (3.2cm × 3.2cm; tulobuterol patch) has been developed in Japan. When applied to the skin of the chest, back, or upper arms once daily, the tulobuterol patch exerts continuous bronchodilatory effects by maintaining stable blood concentrations of tulobuterol for 24 hours.[4,5] The patch may be beneficial for patients who cannot inhale bronchodilators because of severe cough and sputum, severe respiratory problems, or decreased level of consciousness. Although great effort is needed in instructing patients in the proper inhalation technique, to ensure therapeutic effects and to improve compliance with the use of inhaled bronchodilators, such instruction is not necessary for the patch preparation, suggesting that good compliance with treatment can be expected with the tulobuterol patch. In addition, the patch can be removed easily at any time after application to discontinue further transdermal absorption of the remaining tulobuterol molecules if patients experience adverse effects.

In the GOLD guidelines, long-acting β_2-agonists (LABAs) are defined as β_2-agonists that are efficacious for at least 12 hours.[1] Accordingly, the sustained-release patch formulation of tulobuterol can be categorized as long-acting, and maintains effective therapeutic drug concentrations for 24 hours when applied once daily. Inhaled salmeterol is one of the most commonly prescribed LABAs in many countries, and twice-daily inhalation has been shown to improve signs/symptoms and quality of life (QOL) in patients with stable COPD.[6-9] Notably, in a double-blind study conducted in Japan, the tulobuterol patch was demonstrated to be more safe and effective, compared with oral procaterol, in patients with asthma.[10] In a separate double-blind comparative study of inhaled salmeterol and oral procaterol, salmeterol was reported to be superior to procaterol in terms of safety and efficacy in patients with bronchial asthma.[6] The two studies had similar baseline characteristics including patient demographics, study design, and study timelines as well as similar rates of clinical improvement in patients treated with procaterol.[6,10] Accordingly, these two studies conducted in Japan have shown that both inhaled salmeterol and tulobuterol patch improves respiratory function and symptom scores significantly, compared with procaterol tablets, in patients with bronchial asthma.[6,10]

In the present study, we investigated the efficacy and safety of the tulobuterol patch in patients with stable COPD by evaluating its effects on signs/symptoms, QOL, and respiratory function in comparison with salmeterol, a drug with sufficient positive evidence for efficacy and safety, in the treatment of stable COPD.[11-18]

Materials and Methods

Study Design

A total of 17 institutions belonging to the BAREC Research Group participated in the present investigation. In this parallel-group study, patients with stable COPD were randomly allocated to treatment with the tulobuterol patch (Hokunalin® [1]) or salmeterol (Serevent® Diskus). Patients received tulobuterol 2mg applied as a patch 3.2cm × 3.2cm in size, once daily in the evening, to the skin of the chest, back, or upper arm or inhaled salmeterol 50μg twice daily, in the morning and evening. A placebo arm was not set up for legal and ethical reasons.

Patients

Patient inclusion criteria were: (i) a clinical diagnosis of relatively stable COPD over a period of 1 month with symptoms (mainly exertional dyspnea); (ii) FEV_1 (post inhalation of a short-acting β_2-agonist)/FVC <70% predicted, and a postbronchodilator FEV_1 <80% predicted[19] with albuterol (salbutamol) or procaterol corresponding to stage II and stage III COPD in the GOLD guidelines; and (iii) male or female ≥40 years of age. The following patients were excluded from the study: (i) patients with major complaints related to bronchial asthma; (ii) patients receiving home oxygen therapy or with respiratory failure; (iii) patients

1 The use of trade names is for product identification purposes only and does not imply endorsement.

receiving oral corticosteroids; (iv) patients with a history of hypersensitivity to the tulobuterol patch or salmeterol; (iv) patients with a dermatological disorder, including atopic dermatitis, for whom treatment with patch preparations was considered inappropriate; (v) patients with hyperthyroidism, hypertension, heart disease, or diabetes for whom treatment with β_2-agonists was considered inappropriate; (vi) women who were or were suspected to be pregnant, breast-feeding or who desired to become pregnant during the study period; and (vii) other patients for whom participation in the study was considered inappropriate by the investigator. The study protocol was approved by the ethics committee of each participating institution. Written informed consent was obtained from all patients prior to initiation of the study.

Study Protocol

After a 2-week run-in period patients were randomly allocated to treatment with the tulobuterol patch or salmeterol for 12 weeks. Patients who had been receiving β_2-agonists prior to participation in this study were requested to discontinue them at the beginning of the run-in period. New use of any bronchodilator other than the study drugs was not allowed during the run-in and treatment periods. Other drugs that had been used for the treatment of COPD such as inhaled anticholinergics (ipratropium or oxitropium), oral theophylline, inhaled corticosteroids, and expectorants were allowed throughout the study period without changes in the dosage regimen. In addition, on-demand use of inhaled short-acting β_2-agonists to improve COPD symptoms was permitted throughout the study period.

Spirometry was performed at the end of the run-in period and at week 8 during the treatment period, between 14:00 and 18:00h, when the concentration of tulobuterol in serum was at trough level.[10] Patients were instructed to record morning and evening PEF, the use of short-acting β_2 agonists, and subjective symptoms (expectoration, cough, wheezing, activities of daily living, and sleep at night) in a patient diary. The latter had a four-rank scale (0–3) which was used to rate the number of expectorations, ease of expectoration, coughing, and wheezing, and a five-rank scale (0–4) to score activities of daily living[6,10] and quality of sleep at night. PEF was measured using a Mini-Wright peak flow meter (low-range version; ClementClarkInternational Ltd, Harlow, Essex, UK). Patients were instructed to measure PEF two or three times during each session and to record the highest result in the patient diary. Dyspnea and QOL were evaluated at the end of the run-in period and at weeks 4, 8, and 12 during the treatment period, using the Medical Research Council (MRC) Dyspnea Scale and the St George's Respiratory Questionnaire (SGRQ), respectively. The SGRQ consists of 50 disease-specific questions classified into three domains (symptoms, activity, and impact). Total SGRQ score was calculated as the sum of scores for each domain, with a decrease in score assessed as improvement; a change of ≥4 units was considered clinically significant. Occurrence of adverse drug reactions was monitored throughout the study period based on entries in patients' diaries.

Statistical Analysis

Comparisons of data before and after treatment were made using the two-tailed, paired t-test. Between-group comparisons were performed using the two-tailed, unpaired t-test. Values are presented as mean ± standard deviation unless otherwise specified. pP-values <0.05 (two-tailed) were considered to indicate statistical significance.

Results

A total of 119 patients were enrolled in this study, with 59 and 60 patients randomly allocated to tulobuterol patch and salmeterol, respectively. Efficacy of the study drugs was evaluated in 92 patients (46 patients each in the tulobuterol and salmeterol groups). Sixteen patients (nine and seven patients, treated with tulobuterol and salmeterol, respectively) were excluded from analysis because postbronchodilator FEV_1 was not measured, for personal reasons, during the run-in period. Ten patients (three and seven patients treated with tulobuterol and salmeterol, respectively) did not meet the criterion of a postbronchodilator FEV_1/FVC of <70% and a postbronchodilator FEV_1 of <80% predicted; one patient in the tulobuterol group who used tulobuterol tape during the run-in period were also excluded from analysis. Compliance with study drugs was evaluated in 78 of 119 patients (38 and 40 patients, in the tulobuterol and salmeterol groups, respectively) based on entries in patients' diaries; 40 patients who did not keep diaries adequately and the one patient who used tulobuterol tape during the run-in period were excluded from compliance analysis.

Demographical Data

Demographic characteristics and baseline data were similar in the two treatment groups with no significant differences (table I). Many patients (>70%) in both groups had been using bronchodilators such as inhaled anticholinergics and slow-release oral theophylline before participating in this study; the percentage of such patients did not differ significantly between treatment groups. Similarly, the severity of COPD was similar between treatment groups; 67.4% and 50.0% of patients in the tulobuterol and salmeterol groups, respectively, had stage III COPD (GOLD classification).

Table I. Demographical data and patient characteristics

Variable	Tulobuterol patch (n = 46)	Salmeterol (n = 46)	P-value[a]
Age (y)	69.7 ± 7.4	71.6 ± 7.3	0.1156[b]
Sex (% patients)			
male	91.3	95.7	0.6768
female	8.7	4.3	
Smoking (% patients)			
current smoker	26.7	23.9	1.000
ex-smoker	73.3	73.9	
non-smoker	0	2.2	
Duration of COPD (% patients)			
≥5y	34.8	39.1	0.8660
<5y	39.1	32.6	
unknown	26.1	28.3	
BMI (kg/m^2)	21.6 ± 3.7	20.6 ± 2.7	0.1273[b]
Severity (% patients)			
stage II	32.6	50.0	0.0929[c]
stage III	67.4	50.0	
Dyspnea score (MRC)	2.32 ± 0.96	2.46 ± 0.94	0.4771[b]
Xanthine (% patients)	41.3	54.3	0.1739
Anticholinergics (% patients)	65.2	69.6	0.3529
Xanthine or anticholinergics (% patients)	78.3	84.8	0.5921

a Fisher's exact test.

b Paired t-test.

c Wilcoxon rank-sum test.

BMI = body mass index; **MRC** = Medical Research Council.

Spirometry

Baseline FVC, FEV_1, FEV_1/FVC, FEV_1 % predicted, morning PEF, and evening PEF showed no significant difference between treatment groups (table II). After 8 weeks of treatment, mean FVC values increased significantly by 0.12 and 0.15L in the tulobuterol patch and salmeterol groups, respectively, relative to baseline ($p < 0.05$): the increment in FVC did not differ significantly between treatment groups (figure 1). Mean FEV_1 % values after 8 weeks of treatment were 39mL and 70mL higher than the baseline values in the tulobuterol patch and salmeterol groups, respectively; significant improvement in FEV_1 was noted in the salmeterol group ($p < 0.05$) but not in the group treated with the tulobuterol patch ($p = 0.1620$) [figure 2]. However, no significant differences were observed in FEV_1 ($p = 0.4366$) or FEV_1 % predicted ($p = 0.4733$) between treatment groups after 8 weeks of treatment (table II).

Peak Flow Rates

There were no significant between-group differences in the morning and evening PEF rates at baseline (table II). Both groups exhibited improvement in morning and evening PEFs throughout the treatment period; improvement was statistically significant from week 1 to the end of the study period ($p < 0.05$), but there were no significant differences in PEF at any time point of the treatment period between treatment groups (figure 3).

Symptom Scores

Mean COPD symptom scores during the run-in period were: number of expectorations (7.27 ± 5.40 and 6.82 ± 5.71 in the tulobuterol and salmeterol groups, respectively); ease of expectoration (4.68 ± 5.20 and 3.71 ± 3.81); cough (6.11 ± 5.82 and 6.42 ± 5.63); wheezing (3.80 ± 4.47 and 3.24 ± 4.04); activities of daily living (ADL) (10.80 ± 6.60 and 11.74 ± 6.71); and sleep (1.56 ± 3.20 and 1.71 ± 3.61, respectively). No significant between group differences were observed during the run-in period.

The ADL score did not differ between the two groups at any time point, whereas within-group comparison before and after treatment revealed significant improvement in the ADL score only, in recipients of tulobuterol. Improvement in the ADL score

Table II. Pulmonary function data before and after 8 weeks of treatment with the tulobuterol patch or inhaled salmeterol (mean ± standard deviation)

Parameter	Tulobuterol			Salmeterol		
	n	baseline	8 weeks	n	baseline	8 weeks
FVC (L)	38	2.52 ± 0.09	2.64 ± 0.10*	42	2.74 ± 0.10	2.89 ± 0.11*
FEV_1 (L)	38	1.09 ± 0.05	1.13 ± 0.05	42	1.19 ± 0.06	1.26 ± 0.03*
FEV_1/FVC (%)	38	43.5 ± 1.67	43.6 ± 1.49	42	43.6 ± 1.38	43.5 ± 1.34
FEV_1 (% predicted)	38	42.5 ± 2.02	43.8 ± 1.87	42	44.5 ± 1.92	47.0 ± 2.01*
Morning PEF (L/min)	33–42	200.7 ± 10.9	222.0 ± 13.4	33–42	215.1 ± 11.5	237.8 ± 13.7
Evening PEF (L/min)	34–41	210.3 ± 10.4	230.4 ± 12.7	34–41	229.6 ± 12.5	246.2 ± 14.0

* $p < 0.05$ vs baseline (paired t-test).

was significant relative to placebo at weeks 1, 5, 6, and 10 of treatment in patients treated with tulobuterol relative to placebo ($p < 0.05$) [figure 4a]. Similarly, the sleep score was significantly better relative to baseline at weeks 11 and 12 in recipients of tulobuterol ($p < 0.05$), but no significant improvement from baseline was observed in recipients of salmeterol. Between-group comparison of sleep scores revealed significant differences at weeks 7, 8, 11, and 12 of treatment, with better symptom scores at night in the tulobuterol group (figure 4b).

Dyspnea

Baseline MRC dyspnea scores were 2.32 ± 0.96 and 2.46 ± 0.94 in the tulobuterol (n = 46) and salmeterol (n = 46) groups, respectively (p-value for between-group difference was not significant). Dyspnea scores tended to improve with treatment in both groups, exhibiting significant improvement, relative to baseline, by week 12 of treatment (−0.26 and −0.46 for tulobuterol and salmeterol, respectively). Between-group comparison of dyspnea score, however, revealed no significant differences at any point in time.

Quality of Life

The tulobuterol group exhibited significant improvement in total SGRQ scores relative to baseline, whereas the salmeterol group did not (figure 5). Improvement at week 8 (−4.66 score, change >4U) was statistically and clinically significant ($p = 0.0078$). Domain analysis revealed that significantly better scores in both symptoms and impact domains contributed to the overall improvement of total SGRQ in the tulobuterol group. Symptom scores were significantly improved at all time points in the treatment period (−6.94, −12.00, and −11.71 units at weeks 4, 8, and 12, respectively, $p < 0.05$) in recipients of tulobuterol. However, in the salmeterol group SGRQ scores were not significant relative to baseline at any given time point (−3.81, −0.65, and −4.04 units, at 4, 8, and 12 weeks, respectively). Between-group analysis of the SGRQ scores demonstrated significantly better improvement at week 8 in the tulobuterol compared with the salmeterol group ($p < 0.01$). This was largely due to substantial improvement in 'morning wheezing' in tulobuterol recipients. A significant improvement in impact scores relative to baseline, was demonstrated at week 8 (−3.72 units) in the tulobuterol group ($p < 0.05$); the salmeterol group showed no significant improvement in impact scores at any given timepoint. Neither treatment group showed significant improvement in activity scores at any given timepoint.

Compliance

Compliance with the study regimen was significantly better in recipients of the tulobuterol patch (n = 38) compared with salmeterol (n = 40; 98.5 ± 0.72 vs 94.1 ± 1.72%) based on entries in patients' diaries submitted with authorized signature.

Exercise Endurance

In a subgroup analysis of the 6-minute walk test, performed in 11 of the 92 patients, the distance of the 6-minute walk at week 8

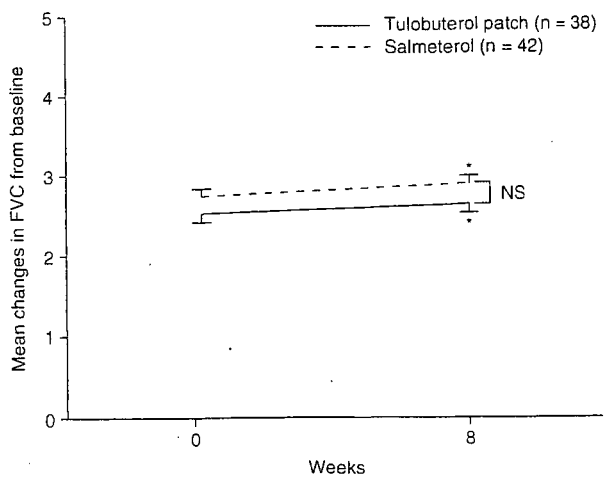

Fig. 1. Mean changes in FVC from baseline after 8 weeks of treatment with the tulobuterol patch or inhaled salmeterol (* $p < 0.05$ vs baseline; paired t-test). Between-group difference was not significant (NS) [unpaired t-test].

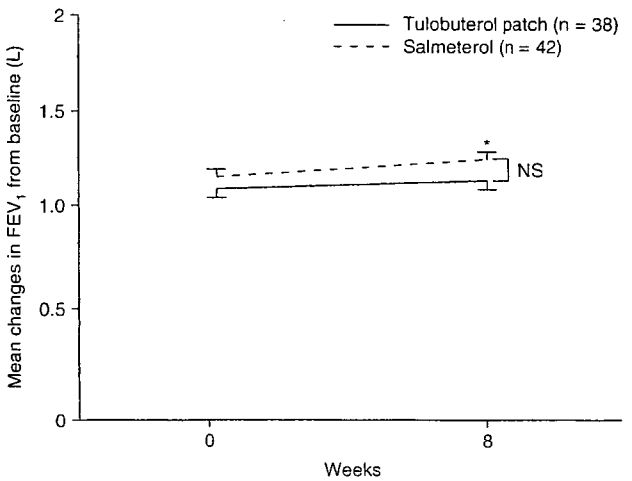

Fig. 2. Mean changes in FEV_1 from baseline after 8 weeks of treatment with the tulobuterol patch or inhaled salmeterol. The mean changes in FEV_1 relative to baseline was significant in recipients of salmeterol (* $p < 0.05$, paired t-test). Between-group difference was not significant (NS) [unpaired t-test].

of treatment (446.0 ± 57.6m, n = 4) was significantly longer than the baseline value (355.0 ± 66.1m) in recipients of tulobuterol ($p < 0.05$), while the distance at week 8 in the salmeterol group (390.0 ± 22.9m, n = 7) did not differ significantly from the baseline value (368.1 ± 31.1m). Tulobuterol thus exhibited better results than salmeterol, although the numbers of patients participating in this test were limited.

Adverse Events

No serious adverse events were reported. In the tulobuterol group, six events of contact dermatitis and one each of hand tremor, numbness, increase in serum creatinine phosphokinase, and abnormal hepatic function were observed. In the salmeterol group, swelling of the upper lip and pharyngeal discomfort were observed in one patient each.

Discussion

Inhaled long-acting bronchodilators are recommended for use in the pharmacotherapy of stable COPD, and salmeterol has been found to be beneficial for this purpose in many studies.[11-18] Sustained-release tulobuterol in a patch formulation has several unique characteristics that make its clinical use beneficial and attractive; it is not dependent on a proper inhalation technique, it can be even used in patients who are unconscious, and is therapeutically effective for 24 hours when administered once daily. In the present study, the tulobuterol patch was as effective as salmeterol in improving pulmonary function, symptom scores, and dyspnea in patients with asthma. Patients treated with tulobuterol exhibited improvements in FVC as well as morning and evening PEFs that were similar to those observed in patients treated with inhaled salmeterol. Although improvement in FEV_1 relative to baseline at week 8 was significant in the salmeterol but not in the tulobuterol group, the between-group difference in FEV_1 was not significant at this timepoint.

The total SGRQ score, in particular the symptom and impact domains, were significantly improved relative to baseline in recipients of the tulobuterol patch while no such improvement was observed in recipients of salmeterol. The lack of a significant effect of salmeterol on QOL in patients with COPD observed in this study, is supported by results from other studies. In a study of

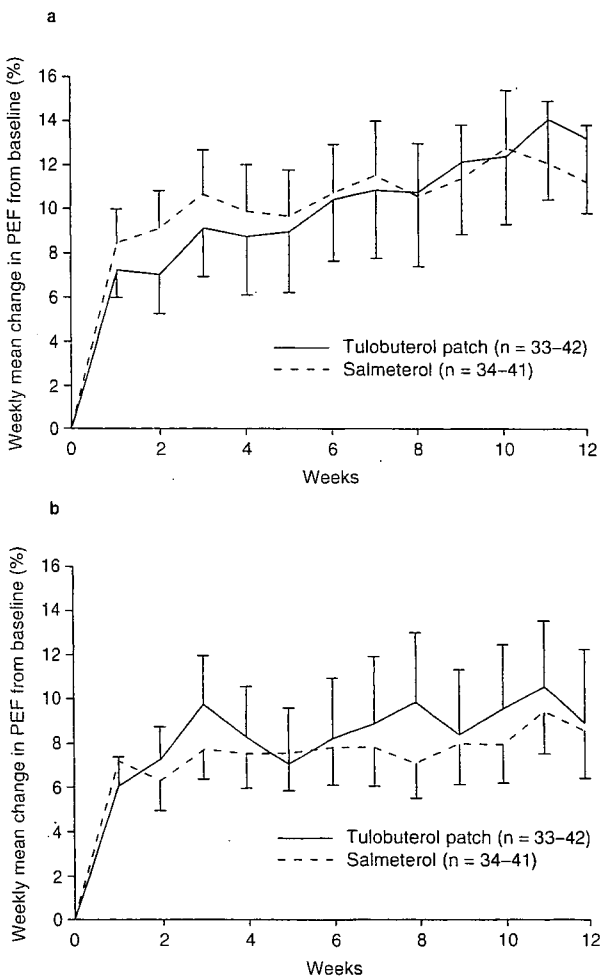

Fig. 3. Serial changes in PEF (mean ± SEM [standard error of the mean]) over a 12-week treatment period with the tulobuterol patch or inhaled salmeterol in patients with stable COPD. Mean change (%) of PEF in (**a**) morning and (**b**) evening compared with baseline mean PEF values. Both tulobuterol and salmeterol improved morning and evening PEF significantly at all timepoints examined during the treatment period compared with baseline PEF ($p < 0.05$, paired t-test). There were significant differences in the degree of improvement in PEF between the tulobuterol and salmeterol groups (unpaired t-test).

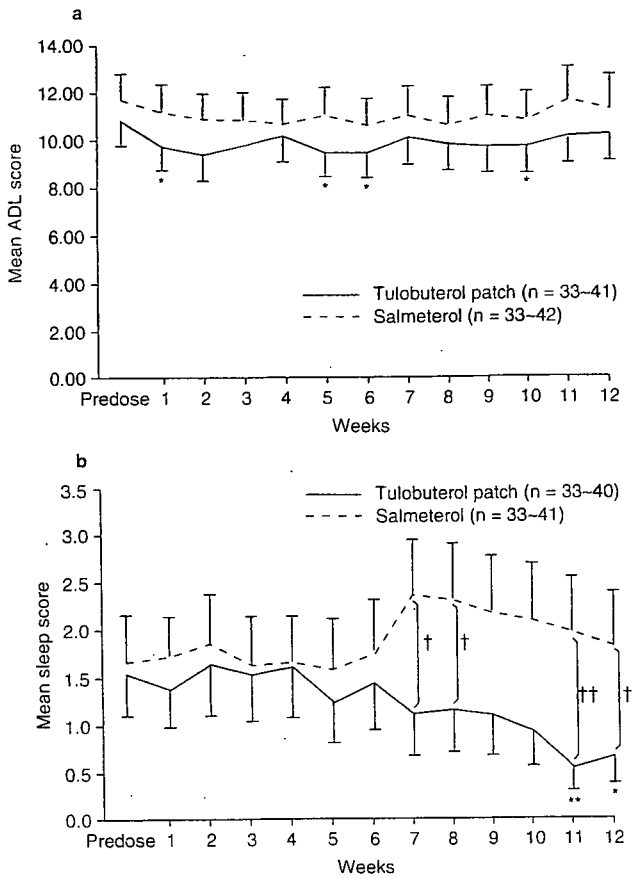

Fig. 4. Serial changes in mean symptom scores (mean ± SEM) over 12-week's treatment with the tulobuterol patch or inhaled salmeterol. Serial changes in (**a**) mean score for activities of daily living [ADL] and (**b**) sleep score. Scores for ADL and sleep, rated on a five-rank scale (0–4), were obtained from patient diaries: lower scores indicate better ADL and quality of sleep. The ADL scores did not differ significantly between treatment groups at any given timepoint during the treatment period (unpaired t-test). However, within-group comparison demonstrated significant improvement in ADL relative to baseline at several timepoints in recipients of tulobuterol alone. The sleep score demonstrated significant improvement at weeks 7, 8, 11, and 12, in tulobuterol compared with salmeterol recipients. * $p < 0.05$, ** $p < 0.01$ vs baseline (paired t-test); † $p < 0.05$, †† $p < 0.01$ vs salmeterol (unpaired t-test).

COPD patients receiving inhaled salmeterol 50 or 100μg twice daily or placebo for 16 weeks, Jones and Bosh[20]reported an improvement of the total SGRQ and impact scores in COPD patients receiving salmeterol 50μg twice daily but not 100μg twice daily. Similarly, Donohue et al. [11]reported a clinically significant improvement in SGRQ (by >4 units) in COPD patients receiving tiotropium but not inhaled salmeterol for a period of 6 months. The reasons for the superiority in improvement of QOL with tulobuterol compared with salmeterol in the present study and the two studies noted above are not entirely clear; it may be related to the route of drug administration. The transdermal patch preparation delivers the drug into the systemic circulation, hence could influence endurance performance. Alternatively, salmeterol, delivered via a breath-actuated device in this investigation, may not have reached the peripheral airways efficiently, the region most severely affected in patients with COPD. In a study by Laube et al.[21] in which eight patients with asthma inhaled a saline aerosol labeled with 99 mTc sulfur colloid, aerosol reached the peripheral airways of the lungs in a patient with and a patient without obstructive airway disease, respectively. Given the limited distribution of inhaled aerosol in the lung, the superiority of improvement in symptoms and QOL in COPD patients treated with tulobuterol is not surprising. The longer length of action of tulobuterol, up to 24 hours after administration, may also have been responsible in part for achieving better results related to QOL compared with salmeterol. Further controlled trials involving larger patient numbers are needed to examine the effects of tulobuterol in improving QOL in patients with COPD.

Absence of attenuation of pharmacological effects is an important requirement for drugs used in the treatment of chronic disease. It is believed that partial β_2-agonists are less likely to induce tolerance to bronchodilation than full β_2-agonists in patients receiving these drugs for a long period of time.[22,23] However, Kume et al.[24,25] reported that even partial agonists may induce tolerance when β_2 receptors are exposed to these drugs at high concentrations. When Kume studied the response of guinea pig tracheal smooth muscle, that was pre-exposed to three long-acting β_2-agonists, formoterol, salmeterol, and tulobuterol differing in intrinsic activity, to the short-acting β_2-agonists (procaterol and albuterol tulobuterol – the drug with the lowest intrinsic activity – did not interfere with the effects of procaterol and albuterol and caused less desensitization than the other two long-acting

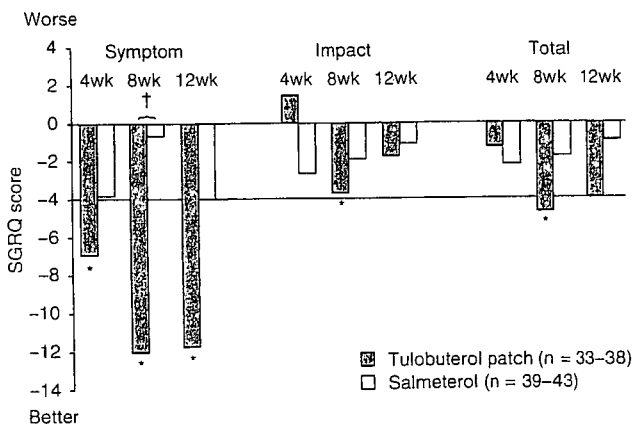

Fig. 5. Changes in St George's Respiratory Questionnaire (SGRQ) score during 12 weeks' treatment with the tulobuterol patch and salmeterol in patients with stable COPD. SGRQ total score, symptoms scores, and impact scores were calculated at weeks (wk) 4, 8, and 12 during the treatment period, and changes from baseline are presented. * $p < 0.05$ vs baseline (paired t-test); † $p < 0.05$ vs salmeterol (unpaired t-test).

β_2-agonists.[25] Although both tulobuterol and salmeterol are classified as partial agonists, some studies have reported the development of tolerance associated with long-term use of salmeterol, whereas no tolerance was found even after continued use of tulobuterol for 1 year in several clinical studies.[24-27] Consistent with these studies, in the present investigation, the patients using tulobuterol exhibited continuous improvement of PEF and subjective symptoms compared with baseline, without the development of tolerance.

Good compliance and safety are also important for drugs used for the long-term management of COPD. In the present study, compliance with the treatment regimen was good for both tulobuterol and inhaled salmeterol, but the overall percentage of compliance was significantly better in the tulobuterol group than in the salmeterol group, supporting the convenience of a once-daily application of a patch preparation for the management of COPD. The safety profiles of the tulobuterol patch and inhaled salmeterol were similar, with patients in both treatment groups experiencing only a few episodes of mild systemic adverse drug reactions that are commonly experienced with β_2-agonists. Although patients using the tulobuterol patch experienced contact dermatitis at the site of administration, it was mild in all cases, controlled easily by changing the site of application on the skin every day, and did not necessitate drug discontinuation. Recently, Eguchi and Hirata[28] reported in their retrospective study that 38 patients with COPD treated with the tulobuterol patch for 3 years maintained effective respiratory function and a good safety profile was noted throughout the treatment period.

Conclusion

In summary, our findings indicate that the once-daily, patch formulation of sustained-release tulobuterol is as effective as the long-acting β_2-agonist salmeterol in the management of stable COPD, with clinically significant effects on QOL and pulmonary function. The tulobuterol patch can be considered as the drug of choice, particularly for COPD patients who cannot inhale drugs because of severe pulmonary dysfunction, dementia, or for other reasons.

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CASE REPORT

Angioimmunoblastic lymphadenopathy with dysproteinaemia accompanied by pleural effusion

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Angioimmunoblastic lymphadenopathy with dysproteinaemia accompanied by pleural effusion
YAMAGATA T, OKAMOTO Y, YAMAGATA Y, NAKANISHI M, MATSUNAGA K, MINAKATA Y, ICHINOSE M. *Respirology* 2005; **10**: 124–127

Abstract: Angioimmunoblastic lymphadenopathy with dysproteinaemia (AILD) is a rare lymphoproliferative disorder characterized by systemic lymphadenopathy, hepatosplenomegaly, loss of body weight, fever, skin eruption, and polyclonal hypergammaglobulinaemia. Occasionally, pulmonary involvement, including pleural effusion, has also been observed. Two cases of AILD accompanied by pleural effusion are reported here. When thoracentesis was performed, an exudative effusion was obtained and there was an increase in soluble interleukin-2 receptor and immunoglobulin G, A, and M in the pleural fluid. Cytologically, atypical plasma cells, and T-cell predominant lymphocytes were also present. These findings are likely to be characteristic of pleural effusions associated with AILD and may prove to be a useful marker for diagnosis.

Key words: angioimmunoblastic lymphadenopathy with dysproteinaemia, atypical plasma cells, pleural effusion, soluble interleukin-2 receptor, thoracentesis.

INTRODUCTION

Angioimmunoblastic lymphadenopathy with dysproteinaemia (AILD) is a rare lymphoproliferative disorder that was established for the first time by Frizzera in 1974.[1] Clinically, AILD is characterized by systemic lymphadenopathy, hepatosplenomegaly, loss of body weight, fever, skin eruption, and polyclonal hypergammaglobulinaemia, and is usually seen in an elderly person. In some reports, pulmonary involvement, including pleural effusion, has also been described.[2,3] However, the characteristics of pleural effusions associated with AILD have not been reported. We describe two cases of AILD accompanied by pleural effusions and discuss the pathological features of the pleural fluid.

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CASE REPORT

Case 1

A 66-year-old man with fever, mild cough and sputum, had an infiltrative shadow and a pleural effusion on CXR (Fig. 1a). On examination, he had widespread lymphadenopathy and reduced breath sounds in the right chest. Laboratory findings showed severe anaemia (red blood cell count $2.38 \times 10^6/mm^3$; haemoglobin 8.2 g/dL; haematocrit 23.9%); thrombocytopenia (platelets $0.7 \times 10^3/mm^3$); and an increase in the level of total protein (9.2 g/dL), γ-globulin (5.9 g/dL), immunoglobulin (Ig) G (4811 mg/dL), IgA (719 mg/dL), and IgM (2078 mg/dL). The percentage of plasma cells in the white blood cell count was increased to 29.0%. Serum soluble interleukin-2 receptor (sIL-2R) was also increased to 16 467 U/mL (normal range, 145–519 U/mL). Histopathology from a lymph node biopsy showed the effacement of