Table 1 Patient characteristics (mean or mean ± SD).

	Treatment group	
	Tio group	Tulo + Tio group
Patients (n)	39	44
Males (%)	94.9	97.7
Age (years)	69.9 ± 5.5	70.2 ± 7.4
Height (cm)	163.14 ± 7.36	164.46 ± 5.63
Weight (kg)	58.29 ± 7.8	59.84 ± 9.79
Severity by GOLD stage (%)		
II	64.1	41.9
III	35.9	58.1
Current smokers (%)	23.1	31.8
Concomitant medication (%)		
Theophyllines	25.6	36.4
Expectorants	17.9	20.5
Short-acting β_2 agonists	10.3	15.9
Dyspnea (MRC scale)	2.5 ± 0.8	2.6 ± 0.9
Pulmonary function tests[a]		
FVC (L)	2.93 ± 0.66	2.92 ± 0.69
FEV_1 (L)	1.39 ± 0.41	1.20 ± 0.35
FEV_1% (%)	47.6 ± 10.8	42.2 ± 9.50
%FEV_1 (%)	53.7 ± 14.7	45.8 ± 12.8
IC (L)	2.01 ± 0.44	1.94 ± 0.47
PEF[a] (L/min)		
Morning	237.3 ± 80.4	218.4 ± 79.0
Evening	246.2 ± 78.3	225.4 ± 81.5
Quality of life scores[a] (units)		
Symptoms	43.1 ± 19.1	51.8 ± 20.1
Activity	45.0 ± 22.2	49.5 ± 19.9
Impact	19.5 ± 12.3	26.6 ± 14.7
Total SGRQ	31.9 ± 14.3	38.7 ± 13.6

GOLD, Global Initiative for Chronic Obstructive Lung Disease; MRC, Medical Research Council; PEF, peak expiratory flow; SGRQ, St. George's Respiratory Questionnaire.
[a] Before the run-in period (during treatment with bronchodilators).

Tio group, as was the change in impact score, a component of the SGRQ.

According to the GOLD, the international initiative establishing guidelines for treatment of COPD, drug therapy with bronchodilators is regarded as the mainstream treatment. Bronchodilators include long-acting agents such as anticholinergics, β_2-agonists and methylxanthines. These agents have different mechanisms of action, and are used according to the responsiveness and symptoms of individual patients. When symptoms are not well controlled, the combined use of bronchodilators with different mechanisms of action is recommended in view of the main and adverse effects, rather than increasing the dose of a single agent.[1] According to the reports of van Noord and colleagues,[10,15] combination therapy with inhaled tiotropium and inhaled formoterol improves pulmonary function better than monotherapy with each agent, and also improves airflow obstruction, resting hyperinflation, and the frequency of rescue therapy with short-acting β_2-agonists, compared with monotherapy with inhaled tiotropium. We selected anticholinergics because the reversible airway contraction in COPD patients mainly depends on acetylcholine released from the vagal nerve, and realized the clinical usefulness of the combined use of an anticholinergic with a β_2-agonist, which stimulates β_2-receptors in airway smooth muscle. The GOLD recommends the use of an inhaled formulation of bronchodilators for COPD treatment, from the standpoint of efficacy and safety. However, because many COPD patients are elderly, their adherence to inhalation therapy becomes an issue. Therefore, as an option to enhance the treatment effect, a dosage form that allows better patient adherence can be selected if acceptable in terms of safety. Although long-acting anticholinergics for COPD treatment are available only in inhaled formulations, long-term β_2-agonists are available in patch formulations as well as in inhaled formulations.

Transdermal tulobuterol is a β_2-agonist designed to maintain drug levels at constant effective concentrations over a 24-h period when applied once daily. In addition, it has an improved safety profile, with a lower maximum blood drug concentration.[12,13] Tamura and coworkers reported that treatment adherence is better with the patch formulation than with the inhaled formulation.[16] Among long-acting β_2-agonists, inhaled salmeterol in a twice-daily regimen is globally used.[17,18] Recently, we conducted a randomized parallel-group comparison study using inhaled salmeterol and transdermal tulobuterol. The study showed that transdermal tulobuterol, compared with inhaled salmeterol, has an equivalent effect in improving pulmonary function, such as FEV_1, FVC, and morning and evening PEF values, and is significantly superior in

Table 2 Changes in pulmonary function, severity of dyspnea, and quality of life scores (mean ± SD).

Parameter	Tio group			Tio + Tulo group			Comparison between-groups
	Baseline	Week 8	Change	Baseline	Week 8	Change	
FVC (L)	2.94 ± 0.11	3.16 ± 0.11***	0.22 ± 0.05	2.91 ± 0.11	3.18 ± 0.10***	0.28 ± 0.05	$P = 0.5379$
FEV_1 (L)	1.38 ± 0.07	1.52 ± 0.07***	0.14 ± 0.03	1.24 ± 0.06	1.37 ± 0.07***	0.13 ± 0.03	$P = 0.6813$
%FEV_1 (%)	50.5 ± 1.97	55.7 ± 1.84***	5.26 ± 0.91	44.3 ± 1.70	49.1 ± 1.89***	4.80 ± 0.93	$P = 0.6948$
IC (L)	2.02 ± 0.07	2.09 ± 0.08	0.07 ± 0.05	1.96 ± 0.08	2.10 ± 0.07*	0.14 ± 0.06	$P = 0.2837$
Dyspnea (MRC scale)	2.5 ± 0.8	2.1 ± 0.8***	−0.3 ± 0.5	2.6 ± 0.9	2.1 ± 0.8***	−0.5 ± 0.6	$P = 0.3798$
Total SGRQ (units)	31.6 ± 2.36	29.6 ± 2.52	−1.96 ± 1.16	38.4 ± 2.04	32.0 ± 2.22***	−6.48 ± 1.72	$P = 0.0325$

MRC, Medical Research Council; SGRQ, St. George's Respiratory Questionnaire.
*$P < 0.05$, ***$P < 0.001$ (within-group comparison: paired t-test).
Comparison between-groups: Wilcoxon rank sum test.

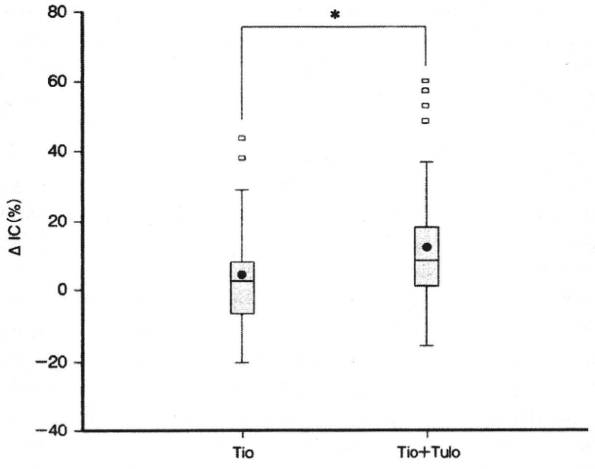

Figure 3 Effect of tulobuterol used in combination therapy on percentage change in IC. The percentage change from baseline at week 8 was assessed in each group. Between-group comparisons were performed for percentage changes. $*P < 0.05$ (Tio group versus Tio + Tulo group); between-group comparison using the Wilcoxon rank sum test DIF, difference.

improving sleep scores. In addition, only transdermal tulobuterol resulted in a significant improvement in QOL (total SGRQ score) from baseline.[14] These findings confirmed that transdermal tulobuterol can replace inhaled salmeterol. Therefore, the use of transdermal tulobuterol, selected as a long-acting β_2-agonist, in combination with inhaled tiotropium for long-term management of COPD, is considered a promising treatment option.

In the present study, in which transdermal tulobuterol was used in combination with inhaled tiotropium, only the Tio + Tulo group showed a significant improvement in IC from baseline, with a significant between-group difference in percentage change in IC. This may be because extensive bronchodilation involving peripheral airways was induced by the combined use of transdermal tulobuterol with inhaled tiotropium, and because the drug was delivered to a wider extent of the airway through the blood circulation.[19,20] In fact, the morning and evening PEF values were significantly increased in both groups, and the percentage change in either morning PEF or evening PEF tended to show more significant improvement in the Tio + Tulo group than in the Tio alone group. These findings suggest that the combined use of transdermal tulobuterol with inhaled tiotropium improves obstruction of the central and peripheral bronchi more than monotherapy with inhaled tiotropium. FEV_1 and PEF are both central airway parameters; theoretically, the FEV_1 should change in the same manner as the PEF. No significant difference in FEV_1 was observed, and only the PEF showed a significant difference, probably because the FEV_1 change was small while, conversely, the change in PEF was large.

We propose the following possible explanations for the additive effects of transdermal tulobuterol and tiotropium. First, the effects of β_2-agonists and anticholinergic agents may be additive because β_2 receptors and muscarinic receptors (M_3) have different mechanisms of action. A recent report[10] described additive effects of β_2-agonists and anticholinergic agents. Second, β_2 receptors and muscarinic receptors (M_3) are both distributed in central and distal airways.[21] Theoretically both agents affect all airways. However, vagal nerve fibers mainly innervate central airways.[22] Therefore stimulated muscarinic receptors (M_3) may be mainly found in central airways. Anticholinergic agents may improve function in central airways rather than peripheral airways because they block cholinergic impulses derived from vagal nerve fibers. Of course, it is also possible that peripheral muscarinic receptors (M_3) are stimulated by acetylcholine of non-neuronal origin.

Regarding the treatment of COPD, it has been reported that improvements not only in pulmonary function but also in QOL are important.[23–26] The results of the present study, in which transdermal tulobuterol was used in combination with inhaled tiotropium, showed significant improvements in the total SGRQ score as well as its activity and impact scores in the Tio + Tulo group compared with the Tio group. The SGRQ is a disease-specific health-related QOL index in COPD patients. The SGRQ consists of 'symptoms', assessing distress owing to respiratory symptoms; 'activity', assessing the effects of disturbance of mobility and physical activity caused by dyspnea; and 'impact', assessing the psychologic and social impact of the disease on daily life and well-being.[27,28] Of the three components, the impact score is highly important as a measurement of health-related

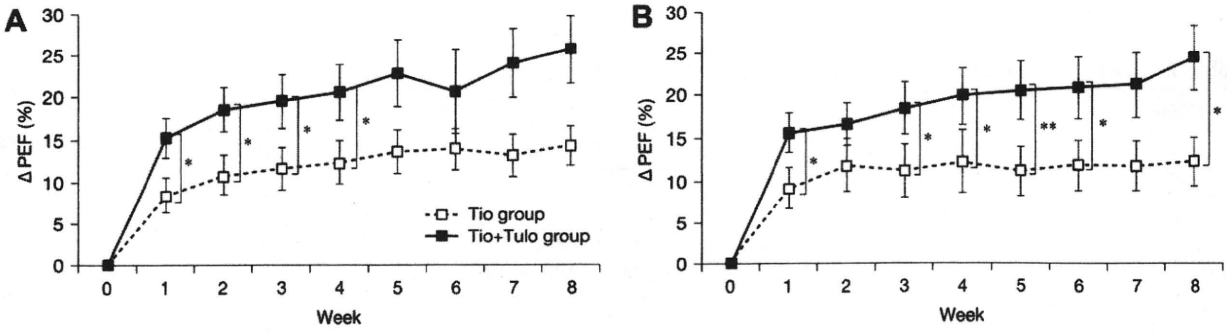

Figure 4 Effect of tulobuterol used in combination therapy on (A) morning and (B) evening peak expiratory flow (PEF). Between-group comparisons were performed for percentage changes. Within-group comparison: Wilcoxon signed rank test, $P < 0.001$ (versus baseline) for all measurements. Between-group comparison: Wilcoxon rank sum test; $*P < 0.05$, $**P < 0.01$ (Tio group versus Tio + Tulo group).

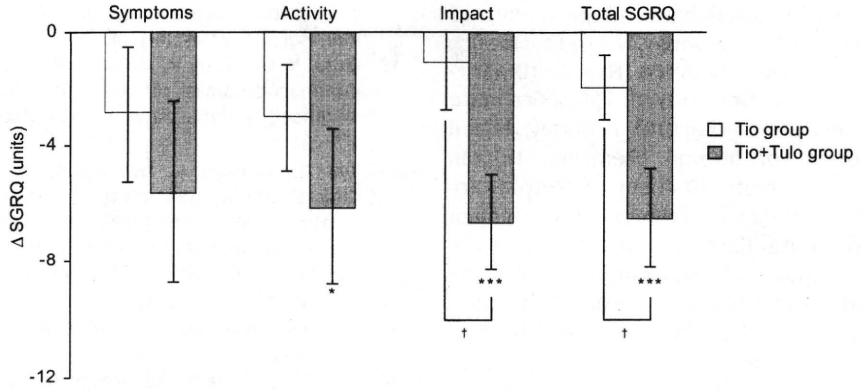

Figure 5 Effect of tulobuterol used in combination therapy on quality of life (St. George's Respiratory Questionnaire, SGRQ, scores). Between-group comparisons were performed to assess changes. Within-group comparison: paired *t*-test; $^{*}P < 0.05$, $^{***}P < 0.001$ (versus baseline). Between-group comparison: Welch's *t*-test, $\dagger P < 0.05$ (Tio group versus Tio + Tulo group).

QOL. Therefore, the improvement in the impact score induced by the combined use of transdermal tulobuterol is significant. The reason for the improvement of QOL by the combined use of transdermal tulobuterol may be that tulobuterol improved peripheral airway obstruction extensively via the systemic circulation, resulting in a reduction in pulmonary hyperinflation.[29,30] Regarding this point, de Torres and associates have reported that the effect of improving IC correlates with a change indicating an improvement in QOL.[31]

Regarding safety, adverse effects were observed in three subjects in the Tio group and one subject in the Tio + Tulo group, but none of these effects were serious. This result suggests that the combined use of transdermal tulobuterol with inhaled tiotropium does not increase the risk of adverse effects.

There are some limitations of the present study. First, because this study was an open-label study, it is possible that biases on the part of patients and/or investigators influenced patient-reported outcomes and other factors in the SGRQ. Second, the imbalance in the baseline QOL scores might have contributed to the difference in the change from baseline between the two groups, because the Tio + Tulo group had more room for improvement. Third, the cardiovascular effects of the two bronchodilators were not objectively assessed. It seems that tulobuterol affected the small airways following systemic delivery. This effect will have to be evaluated in a future study. However, the present findings demonstrate the additive effects of transdermal tulobuterol to inhaled tiotropium. The observed additive effect of transdermal tulobuterol is suggested to be due to a reduction in pulmonary hyperinflation resulting from the drug's effect in improving peripheral airflow obstruction via the systemic circulation. In COPD patients, many of whom are elderly, the combined use of inhaled tiotropium and transdermal tulobuterol is considered to be an ideal combination therapy for COPD.

Table 3 Safety (frequency of adverse events).

No.	Symptom	Tio	Tio + Tulo
1	Palpitation	0	0
2	Increased blood pressure (self-monitoring)	1	0
3	Finger tremor	0	0
4	Sputum	1	0
5	Urticaria	1	0
6	Reduced masticatory force	1	0
7	Dysuria	1	0
8	Pollakiuria	0	0
9	Headache	0	1

Conflict of interest statement

We have no conflicts of interest to declare.

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The funding source had no role.

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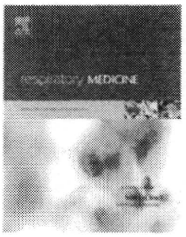

24-h bronchodilator efficacy of single doses of indacaterol in Japanese patients with asthma: A comparison with placebo and salmeterol

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Indacaterol;
Salmeterol

Summary
Background: Indacaterol is a novel, inhaled once-daily ultra-long-acting beta-2 agonist under development as a fixed-dose combination with an inhaled corticosteroid (ICS) for asthma treatment. This study evaluated the 24-h bronchodilator efficacy of indacaterol in Japanese patients with asthma.
Methods: Randomised, placebo-controlled, 5-period crossover study. Patients with persistent asthma (18–75 years, FEV_1 50–85% predicted, ≥12% and 200 mL FEV_1 reversibility) receiving ICS were randomised to double-blind single dose indacaterol 150, 300, or 600 μg or placebo, with open-label salmeterol 50 μg twice-daily for one day in the 5th period. Primary endpoint was FEV_1AUC_{22-24h}.
Results: Of 41 randomised patients (48.8% male; mean age: 47.8 years), 39 completed. All indacaterol doses showed significantly higher FEV_1AUC_{22-24h} than placebo ($P < 0.001$), with treatment–placebo differences of 180, 220, and 260 mL for indacaterol 150, 300, and 600 μg, respectively (salmeterol–placebo difference 170 mL; $P < 0.001$). For individual time-point FEV_1, all indacaterol doses were superior to placebo from 5 min to 24 h post-dose

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($P < 0.001$). Compared with salmeterol, all indacaterol doses were superior from 5 to 30 min ($P < 0.05$); in addition indacaterol 300 μg and 600 μg were superior at a number of subsequent time points. Changes in safety parameters with indacaterol were similar to placebo. All indacaterol doses were well tolerated.

Conclusion: Single dose indacaterol provided sustained 24-h bronchodilation with a faster onset of action than salmeterol and a good overall safety and tolerability profile in Japanese patients with asthma. These results are consistent with data from Caucasian populations.

Introduction

Worldwide, asthma affects people of all ages causing substantial debilitating symptoms and leading to a reduced quality of life.[1,2] Evidence from most studies, including longitudinal surveys, suggests that the prevalence of asthma has increased over the past decades in many parts of the world, including Japan.[3–7] Persistent asthma is most effectively controlled with inhaled corticosteroids (ICSs) administered daily on a long-term basis.[8] For patients with asthma not adequately controlled with a low-dose ICS alone, current treatment guidelines recommend the addition of a long-acting beta-2 agonist (LABA) to the existing ICS therapy.[7–10]

The goal of asthma management is to achieve and maintain control of symptoms – a major obstacle to which has been low patient adherence to medication plans.[11] A key factor contributing to poor adherence is a complicated or multiple treatment regimen, with simplified dosing regimens known to improve compliance.[11,12] Currently available inhaled LABAs (e.g., salmeterol and formoterol) have an approximately 12-h duration of action, necessitating twice-daily (bid) dosing to provide optimal clinical efficacy.[13,14] Thus the availability of a once-daily beta-2 agonist with a 24-h duration of action combined with a once-daily ICS could improve clinical outcomes in asthma by providing increased patient convenience and sustained bronchodilation.

Indacaterol is a novel, once-daily, inhaled, ultra-LABA under development as a fixed-dose combination with ICS for the treatment of asthma. In preclinical studies, indacaterol demonstrated a sustained bronchodilator and bronchoprotective effect, with a fast onset of action (comparable to salbutamol and formoterol) and greater cardiovascular safety margin than formoterol or salmeterol.[15,16] Furthermore, in earlier clinical studies involving mostly the Caucasian population, indacaterol showed effective 24-h bronchodilation, with a fast onset of action and a good overall safety and tolerability profile.[17–21] The present dose-ranging study was the first to investigate the 24-h bronchodilatory efficacy and safety of single doses of indacaterol 150, 300, and 600 μg delivered via a single-dose dry-powder inhaler (SDDPI) in Japanese patients with asthma. Given its similarity to a study conducted in a Caucasian population it helps to evaluate the ethnic sensitivity of the efficacy and safety of indacaterol.[21]

Methods

This was a Phase II, multicentre, randomised, double-blind, placebo-controlled, crossover, dose-ranging study conducted between November 2006 and November 2007 at 11 specialised allergy and respiratory care units in Japan (ClinicalTrials.gov registration no.: NCT00403754).[22] The study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. The study was approved by the institutional review board of each participating study centre, and all patients provided written informed consent before participating in the study.

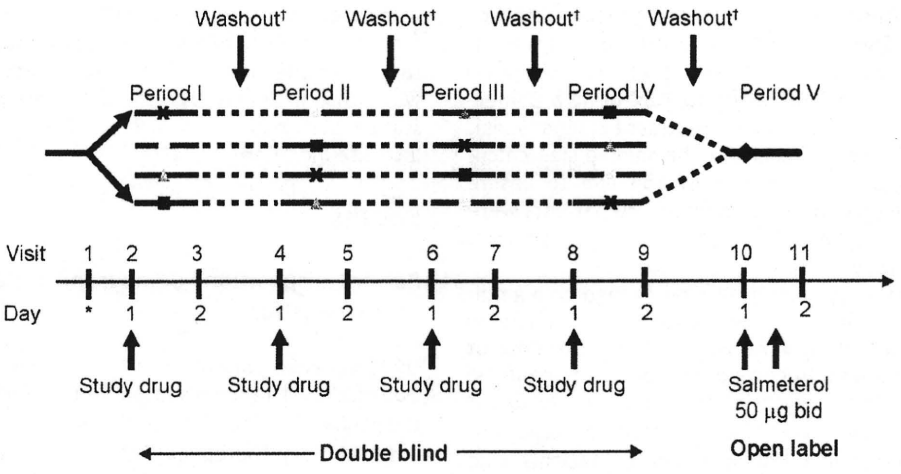
Study population

The study recruited Japanese patients (aged 18–75 years) with a clinical diagnosis of asthma according to the 2006 Japanese asthma prevention and management guideline[7] (FEV_1 between 50% and 85% of the predicted normal value at screening, with ≥12% and 200 mL reversibility in FEV_1 within 30 min after inhalation of salbutamol 200 μg). In addition, patients must have received daily ICS treatment—defined as fluticasone propionate 200–800 μg (or equivalent)—in a stable regimen for at least 4 weeks before the screening visit (Visit 1).

Patients were excluded if they underwent hospitalization or emergency room treatment for an acute exacerbation of asthma in the 6 months before Visit 1, had respiratory disease other than asthma, had respiratory tract infection within 4 weeks before Visit 1, used tobacco products within 6 months before Visit 1, or had a smoking history of more than 10 pack-years. The other exclusion criteria included seasonal allergies that might cause deterioration of asthma; allergy to beta-2 agonists, sympathomimetics, and inhaled medications; ischemic heart disease; arrhythmia; uncontrolled hypertension; type 1 diabetes; cancer; and a prolonged QT interval.

Study design and treatments

The study comprised a 14-day screening period, four double-blind treatment periods (Visits 2–9), and an open-label treatment period (Visits 10 and 11) (Fig. 1). The visits on Day 1 and Day 2 of each treatment period were on consecutive days. Following screening, all eligible patients were randomised equally (at Visit 2), using a validated automated system, to one of four treatment sequences to receive a single dose of indacaterol 150, 300, and 600 μg or placebo via an SDDPI. Patients who completed the double-blind treatment period entered a single-day, open-label treatment period with salmeterol 50 μg bid delivered via the manufacturer's proprietary multidose dry-powder inhaler.



*Visit 1 was a screening visit, approximately 14 days prior to the randomization visit (Visit 2).
†Washout was 14–28 days in duration.

Figure 1 Study design. (-✕-) placebo, (-◆-) salmeterol 50 μg (bid), (——) indacaterol 150 μg, (——) indacaterol 300 μg and (——) indacaterol 600 μg.

During the double-blind treatment periods, the study drug was inhaled between 08:00 and 10:00 AM on Day 1 (Visits 2, 4, 6, and 8). During the open-label period (Visit 10), the first salmeterol dose was inhaled between 08:00 and 10:00 AM, and the second dose was inhaled after spirometry at 12 h post-dose. Each treatment period was separated by a 14–28 day washout period. Treatment allocation was concealed from the patients, investigating staff, and the clinical trial team by using study drugs that were identical in packaging, labelling, appearance, and administration schedule.

Concomitant medication

Other than ICS in a fixed-dose regimen that had been stabilised for at least 4 weeks prior to Visit 1 with the ICS dose remaining unchanged throughout the study, the following asthma treatments were allowed between the treatment periods (following the same regimen as the one before Visit 1): short-acting beta-2 agonists (6 h), LABAs (48 h), xanthine derivatives (7 days), leukotriene antagonists (72 h), and short-acting anticholinergics (8 h). These treatments, however, had to be discontinued no later than the specified time (mentioned within the parentheses above) before Visits 1, 2, 4, 6, 8, and 10, and were not to be recommenced until the completion of the last spirometry evaluations at Visits 3, 5, 7, 9, and 11. Salbutamol was the only rescue medication permitted throughout the study, although visits had to be rescheduled if it was taken within 6 h prior to the first spirometry measurements during that visit.

Assessments

Efficacy and safety were assessed for 24 h post-dose in each treatment period. Efficacy was assessed by spirometry using the same model of spirometer at all sites (MICROSPIRO HI-201®, Nihon Kohden Corporation, Tokyo, Japan). FEV_1, FVC, and $FEF_{25-75\%}$ were determined pre-dose (15 min before inhalation of the study medication), and at 5, 15, and 30 min and 1, 2, 4, 8, 12, 22, 23, and 24 h post-dose. The primary efficacy outcome was the time-standardised area under the curve (AUC) of FEV_1 values measured between 22 and 24 h post-dose (FEV_1AUC_{22-24h}). By using data from up to three time points, this endpoint (which can be considered an average of data from the three evaluations) has the advantage of a smaller standard deviation compared with a single time point, and provides more power for a given number of patients. Further, since data forming this endpoint are from the end of the dosing interval, this endpoint should capture the lowest measured treatment–placebo differences. Secondary efficacy outcomes included percent change in FEV_1 from baseline at each individual time point post-dose; peak FEV_1 (defined as the maximum FEV_1 value recorded between 5 min and 4 h post-dose); standardised FEV_1AUC_{0-24h}; and individual time point FEV_1, FVC, and $FEF_{25-75\%}$.

Safety assessments involved recording of all adverse events (AEs) and serious adverse events (SAEs) in all treatment periods. The occurrence of AEs was sought by non-directive questioning of patients at each visit during the study, and may also have been detected when volunteered by patients during or between visits or through physical examination, laboratory test, or other assessments. In addition, during the double-blind treatment periods, safety was assessed by monitoring—at regular time points in each treatment period—of haematology and blood biochemistry (including serum potassium and blood glucose); urinalysis; and regular assessments of vital signs and ECGs. During the open-label treatment period, with the exception of adverse events no other safety data was collected.

Sample size calculation and statistical analyses

The three main treatment comparisons were indacaterol 150 μg versus placebo, indacaterol 300 μg versus placebo, and indacaterol 600 μg versus placebo. The sample size

calculation was based on the post-dose primary efficacy variable, standardised FEV_1AUC_{22-24h}. A standard deviation (SD) of 283 mL,[23] reversibility threshold of 12%, two-sided significance level of 10% (adjusted to 3.3% using a Bonferroni correction for the three main treatment comparisons), and power of 85% were chosen for the sample size calculation. Assuming a drop out rate of 15% and to ensure balance across the treatment sequences, 40 patients were to be randomised in order to have at least 32 patients completing the study.

The primary efficacy analysis was performed on a modified intention-to-treat (mITT) population, which included all randomised patients who received at least one dose of the study drug. Patients receiving only one treatment did not contribute to the analyses of treatment contrasts but remained in the population for the calculation of treatment means. The safety population included all patients who received at least one dose of the study drug and was used in the analysis of all safety variables. For both populations, the patients were analysed according to the treatment they received.

For the primary efficacy variable, standardised FEV_1 AUC_{22-24h}, treatment differences between each indacaterol dose and placebo were tested using analysis of covariance (ANCOVA), with patient, period, and treatment group modelled as fixed effects and period baseline FEV_1 as a covariate. Adjustment for multiple comparisons was made using a stepwise Dunnett test. ANCOVA models were also used to analyse all the secondary efficacy variables. ANCOVA models were also used to provide between-indacaterol-dose comparisons and comparisons with salmeterol; however, these analyses were regarded as exploratory, because the study was not powered for comparisons between the active treatment groups. The corrected QT (QTc) interval was calculated from the QT and RR intervals using Fridericia's formula (QTcF).[24] All tests of hypotheses used were two-tailed and interpreted at the 10% significance level (as this was a dose-finding study). The data were analysed using SAS statistical software version 8.1 (or higher) for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Patient disposition, demographics, and baseline characteristics

Of 93 screened patients, 41 were randomised to treatment. Out of the 41 randomised patients, 39 (95.1%) completed the study. Two patients (4.9%) discontinued, one due to unsatisfactory therapeutic effect after the second treatment period (having received indacaterol 300 μg and placebo) and the other withdrew consent after the first treatment period (having received indacaterol 150 μg). Table 1 shows the demographics and baseline characteristics of the patients in the study, all of whom were Japanese.

Efficacy

Indacaterol at all doses resulted in a statistically superior ($P < 0.001$) adjusted mean standardised FEV_1AUC_{22-24h} compared to placebo (Fig. 2), with treatment–placebo differences of 180 (90% CI: 120, 250), 220 (150, 280), and 260 (190, 320) mL for the 150, 300, and 600 μg doses, respectively, compared with a salmeterol–placebo difference of 170 (120, 220) mL. At all time points from 5 min to 24 h post-dose, all indacaterol doses showed

Table 1 Patient demographics and baseline characteristics (safety population).

Parameters	Statistic	Total ($N = 41$)
Age, years	Mean (SD)	47.8 (14.90)
	Range	22–74
Sex		
Male	*n* (%)	20 (48.80)
Female	*n* (%)	21 (51.20)
Weight, kg	Mean (SD)	62.1 (11.50)
BMI, kg/m^2	Mean (SD)	23.3 (3.39)
Duration of asthma, years	Mean (SD)	17.5 (14.60)
	Range	0.1–49
ICS daily dose fluticasone propionate equivalent, μg	Mean (SD)	351.2 (177.65)
	Range	200–800
FEV_1 (pre-bronchodilator) at screening, L	Mean (SD)	2.01 (0.66)
% predicted FEV_1 (pre-bronchodilator) at screening	Mean (SD)	67.3 (9.87)
	Range	50.5–84.2
FEV_1 (post-bronchodilator) at screening, L	Mean (SD)	2.4 (0.79)
FEV_1 reversibility*	Mean (SD)	20.1 (7.90)
Smoking history		
Non-smokers	*n* (%)	29 (70.7)
Ex-smokers	*n* (%)	12 (29.3)
Smoking history, pack-years	Mean (SD)	4.3 (2.83)

ICS, inhaled corticosteroid; BMI, body mass index.
*% increase in FEV_1 within 30 min after inhalation of salbutamol from FEV_1 before inhalation of salbutamol.

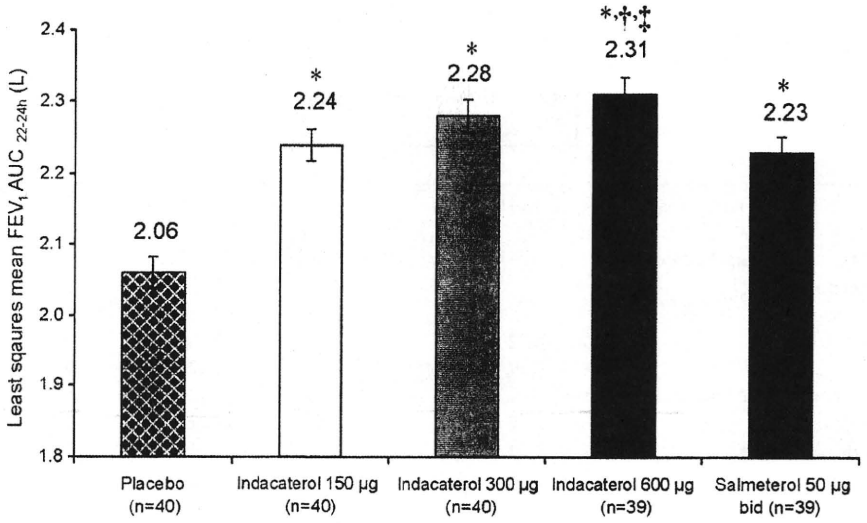


Figure 2 Primary endpoint: standardised $FEV_1AUC_{22\text{-}24h}$ LS mean (SE) (mITT population). AUC, area under curve; SE, standard error; LS, least squares; mITT, modified intent-to-treat. *$P < 0.001$ vs placebo; †$P < 0.05$ vs indacaterol 150 µg; ‡$P < 0.05$ vs salmeterol 50 µg bid.

statistically significant differences ($P < 0.001$) in the adjusted mean FEV_1 as compared with placebo (Fig. 3). In addition, all doses of indacaterol were statistically superior ($P < 0.05$) to salmeterol from 5 min to 30 min post-dose.

Indacaterol resulted in a significantly greater peak FEV_1 compared with placebo ($P < 0.001$) and numerically (150 and 300 µg) or statistically (600 µg) higher peak FEV_1 compared with salmeterol 50 µg bid. All indacaterol doses resulted in significantly higher ($P < 0.001$) adjusted mean FEV_1AUC_{0-24h} than placebo with treatment–placebo differences of 220 (90% CI: 170, 270) mL, 270 (220, 320) mL, and 310 (260, 360) mL for the 150, 300, and 600 µg doses, respectively, and a salmeterol–placebo difference of 200 (150, 240) mL. When compared with salmeterol, the standardised FEV_1AUC_{0-24h} was significantly greater with indacaterol 600 µg (110 mL; $P < 0.001$) and 300 µg (80 mL; $P = 0.0096$), and numerically higher with indacaterol 150 µg (30 mL; $P = 0.34$). All indacaterol doses showed significantly higher FVC, $FEF_{25-75\%}$, and percent change from period baseline in FEV_1 than placebo at all post-dose time points ($P < 0.002$), with profiles similar to those observed for FEV_1 (data not shown). Indacaterol also showed higher FVC, $FEF_{25-75\%}$, and percent change from period baseline in FEV_1 than salmeterol at the majority of the post-dose time points.

Safety

Indacaterol doses were well tolerated, with no deaths or SAEs reported. The overall incidence of AEs during the double-blind treatment phase was 27.5% (11/40), 45.0% (18/40), 40.0% (16/40), and 56.4% (22/39) for placebo and indacaterol 150, 300, and 600 µg, respectively (Table 2). The most commonly reported AE with indacaterol treatment was cough, which was mostly mild in severity and transient in nature, occurring just after inhalation of the study drug.

There were no minimum post-baseline potassium values (below 3.0 mmol/L) with any of the treatments, maximum post-baseline glucose values (above 9.99 mmol/L) were

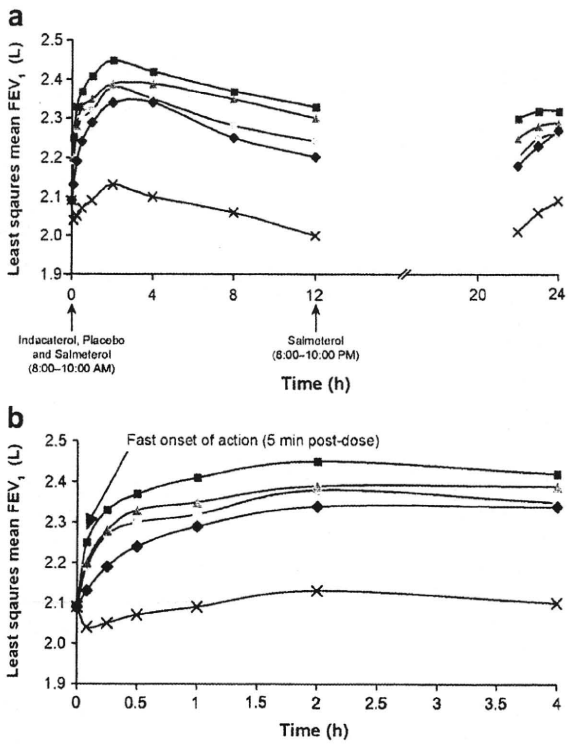


Figure 3 LS mean FEV_1 (a) over 24 h and (b) 0–4 h post-dose (mITT population). LS, least squares; mITT, modified intent-to-treat. (–✕–) placebo, (–◆–) salmeterol 50 µg bid, (——) indacaterol 150 µg, (——) indacaterol 300 µg and (–■–) indacaterol 600 µg. All indacaterol doses superior to placebo ($p < 0.001$) at all post-dose time points and superior to salmeterol ($p < 0.05$) from 5 to 30 min post-dose.

Table 2 Number of patients (%) with AEs and by primary SOC (safety population).

AEs	Indacaterol			Salmeterol	Placebo
	150 μg $N = 40$	300 μg $N = 40$	600 μg $N = 39$	50 μg bid $N = 39$	$N = 40$
			n (%)		
Total number of patients with AEs	18 (45.0)	16 (40.0)	22 (56.4)	2 (5.1)	11 (27.5)
Primary SOC affected					
Respiratory, thoracic, and mediastinal disorders	14 (35.0)	13 (32.5)	15 (38.5)	0	5 (12.5)
Nervous system disorders	2 (5.0)	3 (7.5)	1 (2.6)	1 (2.6)	3 (7.5)
Gastrointestinal disorders	2 (5.0)	1 (2.5)	0	1 (2.6)	0
Infections and infestations	2 (5.0)	0	2 (5.1)	0	1 (2.5)
Investigations	2 (5.0)	0	1 (2.6)	0	1 (2.5)
Musculoskeletal and connective tissue disorders	2 (5.0)	0	1 (2.6)	1 (2.6)	1 (2.5)
Ear and labyrinth disorders	0	1 (2.5)	1 (2.6)	0	1 (2.5)
Hepatobiliary disorders	1 (2.5)	0	0	0	0
Immune system disorders	0	0	1 (2.6)	0	0
Injury, poisoning, and procedural complications	0	0	1 (2.6)	0	0
Psychiatric disorders	1 (2.5)	0	0	0	0
Skin and subcutaneous tissue disorders	0	1 (2.5)	1 (2.6)	0	1 (2.5)

AEs, adverse events; SOC, system organ class.
A patient with multiple AEs on one treatment and under one SOC is counted only once for that organ class and the treatment regardless of the number of AEs.

Table 3 Overall incidence of hypokalaemia, hyperglycaemia, abnormal values of pulse rate and blood pressure, and change from baseline in QTc interval (Fridericia's formula) (safety population).

	Indacaterol			Placebo
	150 μg $N = 40$	300 μg $N = 40$	600 μg $N = 39$	$N = 40$
		n (%)		
Serum potassium: minimum post-baseline value[a]				
Below lower limit of normal range	1 (2.5)	4 (10.0)	3 (7.7)	2 (5.0)
Below 3.0 mmol/L	0	0	0	0
Blood glucose: maximum post-baseline value[a]				
Above upper limit of normal range	22 (55.0)	21 (52.5)	26 (66.7)	20 (50.0)
Above 9.99 mmol/L	0	3 (7.5)	1 (2.6)	0
Pulse rate – maximum post-baseline value[b]				
Above 90 bpm	0	2 (5.0)	0	1 (2.5)
Systolic blood pressure – maximum post-baseline value[c]				
Above 140 mm Hg	7 (17.5)	6 (15.0)	5 (12.8)	5 (12.5)
Diastolic blood pressure – minimum post-baseline value[c]				
Above 90 mm Hg	6 (15.0)	6 (15.0)	4 (10.3)	7 (17.5)
QTc value – (Fridericia's formula)[d]				
Absolute values				
>450 ms (males)	0	0	0	0
>470 ms (females)	0	0	0	0
Change from baseline				
>60 ms	0	0	0	0

[a] The measurement of serum potassium and blood glucose levels were taken at pre-dose, and 15 min, 1 h, 4 h and 24 h post-dose for each treatment.
[b] The measurement of pulse rate was taken at pre-dose, and at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 22 h, 23 h, and 24 h post-dose for each treatment.
[c] The measurement of blood pressure was taken at pre-dose, and 15 min, 1 h, 4 h, 12 h and 24 h post-dose for each treatment.
[d] ECG was taken at pre-dose, 1 h, 4 h, and 24 h post-dose for each treatment.

reported with three patients following indacaterol 300 μg and one patient following 600 μg, none of which were, however, associated with AEs (Table 3).

As summarised in Table 3, the incidence of abnormal values for pulse rate (>90 bpm) and diastolic (>90 mm Hg) and systolic (>140 mm Hg) blood pressure was similar between placebo and the indacaterol treatments, indicating no relationship with indacaterol dose. No clinically significant ECG abnormalities and no abnormal QTcF prolongation (male: QTcF >450 ms; female: >470 ms) or change in QTcF from baseline (>60 ms) were observed with any of the treatments (Table 4).

Discussion

This was the first study to evaluate the 24-h bronchodilator efficacy of single doses of indacaterol 150, 300, and 600 μg in Japanese patients with asthma. This crossover study showed that indacaterol provided sustained 24-h bronchodilation, with a fast onset of action and a good overall safety and tolerability profile. A crossover design was preferred because variations in FEV_1 within patients were expected to be less compared with variations in FEV_1 between patients.

In the present study, the primary efficacy analysis showed that all indacaterol doses were associated with statistically significantly greater ($P < 0.001$) standardised FEV_1AUC_{22-24h} than placebo—a finding consistent with a previous dose-ranging study.[19] Furthermore, the corresponding 90% CIs of the differences between the indacaterol doses and placebo were relatively narrow, indicating the precision of the estimated differences. In terms of comparisons between indacaterol doses, the 600 μg dose was statistically superior to the 150 μg dose for this endpoint, whereas there was no statistically significant difference between indacaterol 150 and 300 μg. In previous studies in patients with asthma in which indacaterol was administered at a dose of 50–600 μg, there were similar separations in efficacy between the lowest and highest indacaterol dose.[19–21,23,25] The current study would suggest that, given the similar efficacy to salmeterol, 150 μg could be the minimal effective dose of indacaterol in asthma – this would, however, need confirming in a more comprehensive dose-finding study. The results of the primary analysis of this study was supported by those of the secondary efficacy analyses, demonstrating that all three indacaterol doses had statistically significant higher peak FEV_1 and standardised FEV_1AUC_{0-24h} compared with placebo ($P < 0.001$). In addition, all indacaterol doses showed notable improvements in FEV_1 at nearly all time points over 24 h compared with placebo—a finding also consistent with earlier studies conducted mostly on the Caucasian population.[18–21,25] These results, together with the 24-h profiles of FVC and $FEF_{25-75\%}$, indicate that indacaterol provided effective and sustained 24-h bronchodilation, which further supports the potential for a once-daily dosing regimen. Given the similar efficacy demonstrated by indacaterol in Japanese and Caucasian patients, this is an indication that indacaterol is likely to be effective in the management of asthma regardless of a patient's ethnicity. This is an important consideration for any bronchodilator, given there is an evidence of divergence in beta-2 adrenoceptor polymorphism haplotype frequency in the ethnic groups[26] In addition, several studies have investigated the polymorphisms of beta-2 adrenoceptor with regard to the bronchodilator response to inhaled beta-2 agonists.[27,28] However, in clinical studies investigating the use of LABAs in COPD patients, there are several reports that demonstrate similar response in Japanese and Caucasian populations.[29,30]

In previous studies in the Caucasian population, indacaterol has shown a fast onset of action, similar to that of salbutamol (a short-acting beta-2 agonist) and faster than salmeterol.[18] The results of our study are also consistent with these findings, as significant improvements in FEV_1 appeared as early as 5 min post-indacaterol dose (the earliest time point evaluated), with all indacaterol doses showing statistical superiority ($P < 0.05$) to salmeterol until at least 30 min post-dose.

The convenience of once-daily dosing together with sustained bronchodilation and a fast onset of action could potentially enable enhanced patient compliance and asthma control compared with twice-daily therapies.[20] Thus indacaterol, when combined with a once-daily ICS, could have an advantage in terms of patient acceptability, convenience, and adherence to therapy. These aspects of indacaterol treatment could not be assessed in the current study, given the single-day dosing study design. Furthermore, salmeterol was administered on an open-label basis in the final treatment period for all patients. This was for practical purposes, and made the study design less complex (with only one type of placebo inhaler required), and it should be noted that the primary comparison was between indacaterol and placebo. However, the inclusion of a LABA active comparator is helpful in the interpretation of the study results.

All indacaterol doses in the present study were well tolerated and showed a good overall safety profile. No patient treated with indacaterol discontinued the study due to AEs. Most AEs reported in this study were expected in this patient population and were similar to those observed in previous studies conducted outside Japan.[17,18,20,31] The incidence of AEs reported in this study was higher with indacaterol treatment than with open-label salmeterol (which was taken by all patients during the final treatment period). This difference in the rate of AEs was due to the higher incidence of cough in patients treated with indacaterol. The majority of cough AEs were mild in severity, occurred just after inhalation of study drug and were transient in nature. Notably, recording of AEs was the only safety assessment performed for salmeterol.

No deaths or SAEs were reported in this study. Given the class effects of beta-2 agonists on serum potassium and blood glucose, these parameters were analysed extensively, and there was no clear relationship between the incidence of hypokalaemia or hyperglycaemia and the indacaterol doses. Furthermore, no major effect on the QT interval and pulse rate was observed with the indacaterol doses.

The overall safety and tolerability profile of indacaterol was consistent with the previous studies that involved repeated indacaterol doses for up to 28 days.[17,27] Further studies investigating the long-term efficacy and safety of

indacaterol in a fixed-dose combination with an ICS are planned.

In conclusion, single doses of indacaterol 150, 300, and 600 μg in combination with an ICS provided superior bronchodilation for 24-h with a faster onset of action compared with salmeterol 50 μg bid in combination with an ICS in Japanese patients with asthma. In addition, indacaterol demonstrated a good overall safety and tolerability profile in this patient population. Therefore, the development of indacaterol in conjunction with an ICS could be a useful treatment option for Japanese patients with asthma.

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Conflict of interest statement

This study was funded by Novartis Pharma AG, Basel, Switzerland. Motoi Hosoe, Benjamin Kramer, Mark Higgins and Charlotte Emery are employees of Novartis. Benjamin Kramer and Mark Higgins holds shares in Novartis. Mitsuru Adachi has previously served on scientific advisory boards of GlaxoSmithKline KK, Nippon Boehringer Ingelheim, Novartis Pharma KK, AstraZeneca KK, Shering-Plough KK, and KYORIN Pharmaceutical Co., Ltd. He has received lecture fees from GlaxoSmithKline KK, Nippon Boehringer Ingelheim, Novartis Pharma KK, AstraZeneca KK, Shering-Plough KK, and KYORIN Pharmaceutical Co., Ltd. and unrestricted grants from GlaxoSmithKine KK, Nippon Boehringer Ingelheim and Novartis Pharma KK. Masakazu Ichinose has previously served on scientific advisory boards of GlaxoSmithKline KK, Nippon Boehringer Ingelheim, Novartis Pharma KK and AstraZeneca KK. He has received lecture fees from GlaxoSmithKline KK, AstraZeneca KK and Nippon Boehringer Ingelheim and unrestricted grants from GlaxoSmithKine KK and Nippon Boehringer Ingelheim. Naruhiko Sugihara, Shigeto Kanada and Michiko Haida have no relevant conflicts of interest or financial disclosures. Naruhiko Sugihara, Shigeto Kanada, Michiko Haida, Masakazu Ichinose and Mitsuru Adachi contributed to the data collection. Motoi Hosoe, Benjamin Kramer and Charlotte Emery were involved in the study design and in overseeing conduct, analysis and interpretation of data. All authors contributed to the development of the manuscript, and approved the decision to submit the manuscript.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

High COPD Prevalence in Patients with Liver Disease

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Abstract

Objective Comorbidities of chronic obstructive pulmonary disease (COPD) have been recognized as an important issue in COPD management. We have reported that patients with liver diseases show a higher prevalence of COPD, but the number of patients with liver diseases was small and the details of liver diseases were not clearly investigated. In this study, we investigated the prevalence of COPD in patients with liver diseases by recruiting a large number of patients, and also investigated was the effect of hepatitis virus infection on COPD prevalence.

Patients and Methods Six hundred sixty-six patients were recruited from 9 primary care clinics and three hospitals. All of these patients were aged 40 years or older with chronic diseases and had not been diagnosed as having respiratory diseases. A spirometry was performed without administration of an inhaled bronchodilator. Airflow limitation was defined as FEV_1/FVC <70%. Underlying diseases were diagnosed by doctors of the clinics or the hospitals.

Results Two hundred fifty-six patients had liver diseases, and 410 did not. Of 410 patients without liver diseases, 37 patients (9.0%) were diagnosed as COPD, and of 256 patients with liver diseases, 35 patients (13.8%) were COPD. When the prevalence was analyzed according to smoking, age and gender, liver diseases showed a significantly high odds ratio (2.10, 95%CI 1.23-3.57, p=0.006), but hepatitis virus infection showed a non-significant tendency toward a high odds ratio.

Conclusion The patients with liver diseases had a significantly high prevalence of COPD. The presence of liver disease might become a useful predictor for the early detection of COPD.

Key words: liver disease, comorbidity, COPD, hepatitis virus

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Introduction

Chronic obstructive pulmonary disease (COPD) is the fifth leading cause of death in high-income countries, and the sixth leading cause of death in nations of low and middle income in 2001 (1). COPD is one of the most important causes of death in most countries. Actually, the mortality of COPD increased from 1970 through 2002 in the United States though the mortality of other diseases declined (2). Respiratory failure is considered the major cause of death in advanced COPD, but comorbidities such as cardiovascular diseases and lung cancer are also major causes of death in mild-to-moderate COPD.

Patients with COPD have been reported to have a higher prevalence of certain comorbid conditions including ischemic heart disease (3), congestive heart failure (3), lung cancer (4), depression (5), osteoporosis (6), hypertension (7) and diabetes mellitus (8), and have an average of 3.7 chronic medical conditions, compared with 1.8 chronic medical conditions for patients without COPD (9). Ischemic heart disease, hypertension and lung cancer are classified as common pathway comorbidities, which are the diseases with a common pathophysiology as, for example, smoking related diseases (10). Congestive heart failure is classified as a complicating comorbidity since it is a condition that arises as a

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complication of COPD (10). The presence of these diseases might affect the treatment and prognosis of COPD. In contrast, depression, osteoporosis and diabetes mellitus are classified as co-incidental comorbidities, which are coexisting chronic conditions with unrelated pathogenesis (10). The presence of these diseases might become a useful indicator for early detection of COPD.

Recently, we reported that COPD prevalence was 10.3% among 474 patients who visited primary care clinics with chronic diseases other than respiratory diseases. In this report, we demonstrated that the patients with liver diseases showed a higher prevalence of COPD (11). However, the number of the patients with liver diseases was small (n=64), and the details of the liver diseases were not clearly investigated, though an interaction between hepatitis virus and the reduction of forced expiratory volume in one second (FEV_1) has been reported (12).

In this study, we investigated the prevalence of COPD in patients with liver diseases by recruiting a large number of patients, and also investigated the effects of hepatitis virus on the COPD prevalence.

Methods

Subject recruitment

The study was conducted in patients who visited nine primary care clinics or three hospitals in Wakayama Prefecture in June 2006 and in July to December 2008. In the first period, the patients were randomly recruited, and in the second period, only the patients with liver diseases were recruited. Patients were selected according to the following criteria: aged 40 or older, with chronic diseases but without having been diagnosed as having respiratory diseases, able to undergo spirometry, and willing to provide written informed consent. The information about age, sex, clinical history, smoking status, underlying diseases, and the detailed diagnoses of liver diseases were obtained from the doctors. The study was approved by the local ethics committee.

Spirometry

Spirometry was performed using a CHESTGRAPH Jr. HI-101 spirometer (Chest MI, Inc., Tokyo, Japan). All subjects performed at least three forced vital capacity (FVC) maneuvers and slow vital capacity (VC) maneuvers according to the recommendations of the American Thoracic Society and European Respiratory Society (13). The highest FEV_1 and FVC values were recorded. The positive criterion for a diagnosis of airflow limitation was a FEV_1/FVC ratio (FEV_1%) of <70%. If obstructive changes were detected, further examinations including questionnaires and chest X-rays were performed. The final diagnosis was made based on symptoms, the presence of allergic history, and chest X-ray using Global Initiative for Chronic Obstructive Lung Disease criteria (10). Patients were diagnosed as asthma when they had symptoms at night or early morning or a history of allergic disease, and as bronchiectasis when they had sputum and bronchial dilatation on their chest X-ray or CT.

Statistical analysis

Relationships between the prevalence of COPD and the liver diseases or details of liver diseases were examined by logistic regression analysis. In the analysis model, the presence of COPD was set as a dependent variable and the presence of liver diseases or details of liver diseases as independent variables. In order to adjust the prevalence according to the amount of smoking (pack-years), age and gender, were set as adjustment factors. We calculated the odds ratio and 95% confidence interval (95% CI) for having COPD. For comparison of the variables between patients with and without liver diseases, Chi-square tests and unpaired t-tests were used. A p value of less than 0.05 was considered statistically significant. The calculations were made with PASW statistics 18 (SPSS Japan Inc., Tokyo, Japan).

Results

Among the enrolled 666 patients who were aged ≥40 years with chronic diseases but had not been diagnosed as having respiratory diseases, 410 were patients without liver diseases and 256 were patients with liver diseases. Gender and body mass index (BMI) was not different between the two groups. The patients with liver diseases were younger than those without liver diseases (without liver diseases 67.9±10.2, with liver diseases: 63.2±9.8), especially the percentage of those aged 70 or older was lower among patients with liver diseases (without liver diseases: 50.2%, with liver diseases: 27.3%; $p<0.001$). The percentages of non-smokers were not different between the two groups, but the percentage of ex-smokers was lower and that of current-smokers was higher in the patients with liver diseases compared to those without liver diseases. In the pulmonary function tests, the values of VC, FVC, FEV_1 were higher and FEV_1% was lower in the group with liver diseases, but the values of FEV_1% predicted were not different between both groups (Table 1). The non-liver disease group was composed of 189 patients (46.1%) of hypertension, 169 (41.2%) of diabetes mellitus, 110 (26.8%) of hyperlipidemia, 61 (14.9%) of cardiovascular diseases, 58 (14.1%) of digestive organ diseases, 17 (4.1%) of cerebrovascular diseases, 29 (7.1%) of malignant diseases, and 46 (11.2%) of other diseases. The liver disease group was composed of 117 patients (45.7%) of type C viral hepatitis, 48 (18.8%) of liver cirrhosis, 27 (10.5%) of hepatocellular carcinoma, 21 (8.2%) of type B viral hepatitis, 19 (7.4%) of autoimmune hepatitis, 17 (6.6%) of nonalcoholic fatty liver disease, 16 (6.3%) of alcoholic hepatitis, 11 (4.3%) of fatty liver disease, and 9 (3.5%) of primary biliary cirrhosis.

Among the 410 patients without liver diseases, 37 had COPD, 3 asthma, and 370 were normal, and the prevalence of COPD was 9.0%. Compared with this, among the 256 patients with liver diseases, 35 had COPD, 2 asthma, 1

Table 1. Patients Characteristics

		without liver diseases	with liver diseases	p-value
Sex [M/F]	(n)	224 / 186	143 / 113	0.757
BMI	(kg/m^2)	23.3 ± 3.4	23.5 ± 3.5	0.404
Age (yr)		67.9 ± 10.2	63.2 ± 9.8	<0.001
40 - 49	n (%)	26 (6.3)	24 (9.4)	0.148
50 - 59		56 (13.7)	60 (23.4)	0.001
60 - 69		122 (29.8)	102 (39.8)	0.007
70 -		206 (50.2)	70 (27.3)	<0.001
Smoking	n (%)			
non		197 (48.0)	125 (48.8)	0.845
ex		137 (33.4)	61 (23.8)	0.009
current		76 (18.5)	70 (27.3)	0.008
Pack-Year(smoker)		44.4 ± 33.0	41.8 ± 32.6	0.483
Pulmonary function test				
VC	(L)	2.89 ± 0.81	3.05 ± 0.84	0.015
FVC	(L)	2.72 ± 0.80	2.97 ± 0.79	<0.001
FEV_1	(L)	2.17 ± 0.65	2.31 ± 0.65	0.007
FEV_1%	(%)	80.0 ± 8.4	77.8 ± 7.5	<0.001
FEV_1 %pred.	(%)	90.8 ± 16.9	91.8 ± 19.6	0.506

BMI: body mass index, VC: vital capacity, FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, FEV_1%: 100*FEV_1/FVC, FEV_1 %pred.: FEV_1 % of predicted value

Table 2. COPD Morbidity

	without liver diseases	with liver diseases
FEV_1/FVC <70%	40/410	38/256
Diagnosis		
COPD	37 (9.0%)	35 (13.7%)
Asthma	3	2
Bronchiectasis	0	1

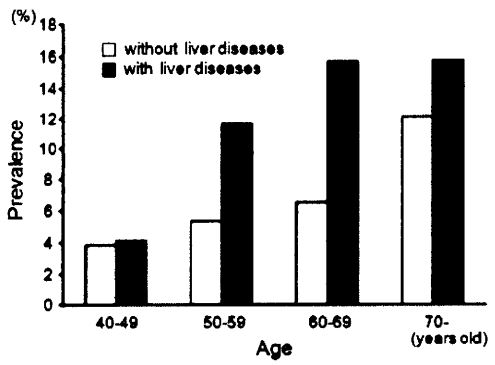

Figure 1. Prevalence of COPD according to age.

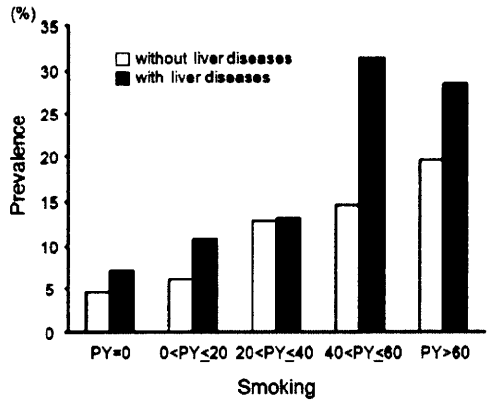

Figure 2. Prevalence of COPD according to smoking. PY: pack year

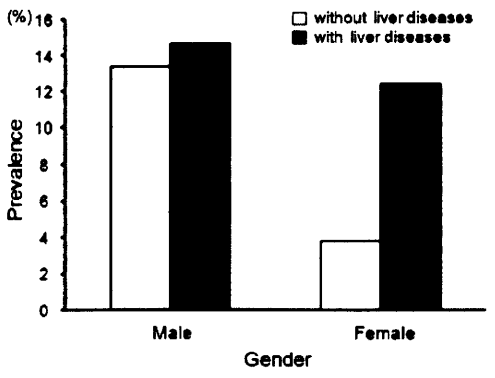

Figure 3. Prevalence of COPD according to gender.

bronchienctasis, and 218 were normal, and the prevalence of COPD was 13.7% (Table 2).

When the prevalence of COPD was calculated according to age, the values were higher in liver diseases for all age groups (Fig. 1). When calculated according to the amount of smoking, the values were higher in liver diseases at any amount of smoking (Fig. 2). When calculated according to gender, the values were higher in liver diseases for both genders (Fig. 3).

The relative risk of COPD in liver diseases was significantly high and the odds ratio was 2.10 (95%CI 1.23-3.57, p=0.006) when adjusted according to smoking, age and gender (Table 3). The relative risks of COPD in patients with hepatitis B virus (HBV) and hepatitis C virus (HCV) were

Table 3. Relative Risk of COPD

	total (n)	COPD (n)	Unadjusted Odds ratio (95%CI)	p-value	Adjusted* Odds ratio (95%CI)	p-value
Liver diseases	256	35	1.60 (0.98 – 2.61)	0.062	2.10 (1.23 – 3.57)	0.006
HBV positive	26	4	1.53 (0.51 – 4.57)	0.447	3.01 (0.93 – 9.72)	0.066
HCV positive	156	22	1.51 (0.88 – 2.59)	0.132	1.69 (0.97 – 2.96)	0.066
Chronic hepatitis	208	27	1.37 (0.82 – 2.28)	0.226	1.86 (1.07 – 3.24)	0.027
Liver cirrhosis	48	8	1.73 (0.78 – 3.86)	0.180	1.67 (0.73 – 3.81)	0.224
HCC	27	7	3.09 (1.26 – 7.59)	0.014	3.10 (1.23 – 7.86)	0.017

HBV: hepatitis B virus, HCV: hepatitis C virus, HCC: Hepatocellular carcinoma,
*: adjusted by smoking, age and gender

high but not significantly, and the odds ratios were 3.01 and 1.69, respectively (HBV; 95%CI 0.93-9.72, p=0.066, HCV; 95%CI 0.97-2.96, p=0.066). The relative risk in chronic hepatitis was significantly high when adjusted according to smoking, age and gender (Table 3).

Discussion

We demonstrated that the patients who regularly go to the medical facilities for liver diseases have a significantly higher prevalence of COPD than those without liver diseases. There were no significant differences between the patients with and those without hepatitis viruses (HBV and HCV) in the prevalence of COPD.

We previously reported that among 474 patients with non-respiratory chronic diseases, the prevalence of COPD was higher in those with liver diseases, though the number of patients with liver diseases was only 64 (11). Similar results have been reported by Fukahori et al, in which the FEV_1% in patients with chronic hepatitis was significantly lower than in patients without liver disease when adjusted according to smoking, age and gender, though the number of the patients with liver diseases was 45 (14). In the present study, we recruited 256 patients with liver diseases and demonstrated that the patients with liver diseases had a significantly higher prevalence of COPD (35 out of 256; 13.7%) than those without liver diseases (37 out of 410; 9.0%) when adjusted according to smoking, age and gender. We also demonstrated that patients with chronic hepatitis had a significantly higher prevalence of COPD. Though the patients with liver cirrhosis did not have a higher prevalence of COPD, the influence of liver cirrhosis on the prevalence of COPD could not be excluded, because the number of patients with liver cirrhosis was small (n=48).

The patients infected with hepatitis viruses (HBV and HCV) did not have a significantly higher prevalence of COPD than those who were not. Kanazawa et al reported that the decline of FEV_1 and diffusing capacity for carbon monoxide in current smokers and ex-smokers were significantly greater in HCV-positive patients than in HCV-negative patients. In addition, the decline of FEV_1 in the interferon (IFN) non-responders did not change during the 3-year follow-up period, but, the decline of FEV_1 in the IFN responders was significantly reduced (12). These findings suggest that chronic HCV infection might be associated with an accelerated decline of lung function and also with COPD. Furthermore, Silva et al. reported that the rate of HCV positivity in COPD patients (10.7%) was significantly higher than in controls (0.41%) (15). In the current study, the relative risks of COPD in the patients with HCV or HBV were not significant but tended to be higher when adjusted according to smoking, age and gender (HBV; n=26, p=0.066, HCV; n=156, p=0.066). These results suggest that hepatitis virus, especially HCV might be associated with COPD.

The precise molecular mechanisms of the relationship between liver diseases and COPD are unclear. However, increased systemic inflammation in liver diseases might be associated with COPD. An elevation of markers of systemic inflammation can frequently be found in patients with COPD (16, 17). The most plausible molecule is interleukin (IL)-8, which is one of the key molecules in the pathogenesis of COPD. Hepatitis C virus infection induces IL-8 production in human endothelial cells (18, 19), and the IL-8 levels are associated with HCV replication (20). The enhanced production of IL-8 is induced via transcriptional activation and mRNA stabilization (18). Furthermore, the core nucleocapsid protein of HCV induces the up-regulation of IL-8 through the enhancement of p38- and gC1qR (21). An elevation of the serum level of IL-8 has also been observed in patients with nonalcoholic steatohepatitis (22) and those with alcoholic liver disease (23).

Though COPD patients have been reported to have a higher prevalence of ischemic heart disease (3), congestive heart failure (3) and lung cancer (4), the prevalence of COPD in cardiovascular diseases or malignancy was not high in the current study. Ischemic heart disease and lung cancer are categorized as common pathway comorbidities since they are smoking-related diseases as is COPD (10). Congestive heart failure is mainly categorized as a complicating comorbidity, since it is easily induced by pulmonary hypertension, a complication of COPD (10). Some patients with these diseases may easily be suspected as having COPD and might have been already diagnosed with COPD. As we recruited patients who had not been diagnosed with respiratory diseases in the current study, the prevalence of COPD in these diseases might not be high.

There were some limitations in this study. First, spirometry was done only in a pre-bronchodilator condition, because

the inhalation of bronchodilators is not allowed for patients without respiratory diseases at primary care clinics. It has been reported that the disease prevalence after the use of a bronchodilator could be 5-50% lower than before (24, 25). Therefore, the prevalence of COPD in both groups might be overestimated. Second, the patients in each group were not age-matched. The patients with liver diseases were younger than those without liver diseases, which might cause higher values of VC, FVC and FEV_1 in the liver disease group. Though the distribution of age was different between the 2 groups, it was higher in patients with liver diseases for all age groups, and the prevalence of COPD was significantly higher in the patients with liver diseases than in those without liver diseases after adjusting according to smoking, age and gender. This suggests that the unmatched distribution of age would not affect the results.

Conclusion

The patients with liver disease had a significantly higher prevalence of COPD than those without liver diseases. The patients with hepatitis virus, though not significantly, tended to have a higher prevalence of COPD. The presence of liver diseases might become one of the useful predictors for the early detection of COPD.

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Statins inhibit matrix metalloproteinase release from human lung fibroblasts

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ABSTRACT: **Pleiotropic effects of statins have been reported to include inhibition of matrix metalloproteinase (MMP) release from macrophages and endothelial cells. We evaluated whether statins would inhibit MMP release from human lung fibroblasts, which play a major role in remodelling processes.**

Monolayer and three-dimensional (3D) collagen gel cultures of fibroblasts were used. Cytokines (tumour necrosis factor-α and interleukin-1α) were used to induce MMP release and mRNA expression. Collagen degradation induced by cytokines and neutrophil elastase (NE) was evaluated by quantifying hydroxyproline. Atorvastatin inhibited MMP-1 and -3 release and mRNA expression in both culture systems. Similar results were obtained with simvastatin and fluvastatin.

In 3D cultures where cytokines also stimulated MMP-9 release, atorvastatin also inhibited MMP-9 release. In 3D cultures, cytokines together with NE induced collagen degradation, which was also inhibited by atorvastatin. The effect of atorvastatin was reversed by mevalonate and geranylgeranyl-pyrophosphate but not by farnesyl-pyrophosphate.

The current data suggest that statins may modulate remodelling processes mediated by fibroblasts by inhibiting MMP release.

KEYWORDS: **Collagen, degradation, fibroblasts, matrix metalloproteinases, statins**

Chronic obstructive pulmonary disease (COPD) is expected to become the third leading cause of death worldwide by the year 2020 [1]. The defining feature of COPD is loss of expiratory airflow that can result from at least two important structural alterations in the lungs, emphysema with its destruction of alveolar wall and loss of lung elastic recoil, and peribronchial fibrosis, which narrows airways. At present, therapy can partially alleviate symptoms, but has relatively little impact on the progressive structural alterations that compromise lung function [2]. Novel therapeutic strategies that have the potential to alter lung structure, therefore, are of interest as possible approaches to alter the long-term natural history of COPD. One type of therapy that is attracting interest for its potential in COPD is the use of statins, which have been associated with reduced acute events and mortality [3–5], as well as with reduced decline in lung function in retrospective database studies [6].

Statins are a class of cholesterol-lowering drugs that inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA). Recently, pleiotropic effects of statins have been reported, including anti-inflammatory and antioxidant effects [7, 8], as well as the inhibition of matrix metalloproteinase (MMP) release from vascular cells and macrophages [9–11]. One potential mechanism for statin efficacy in COPD could be effects on inflammatory cells. Such effects could be a mechanism that could alter both acute events and long-term progression. An alternate mechanism, which could be either independent or interactive, is the effect of statins on MMPs. Through these effects, statins could directly modify lung extracellular matrix (ECM) and, thereby, could modify the alterations in lung structure that compromise lung function.

The MMPs are a large family of proteolytic enzymes [12] that are produced by both inflammatory cells and structural cells in the lung including alveolar epithelial cells and fibroblasts [13]. Fibroblasts are believed to be major cells responsible for maintenance and repair of ECM [14]. This is accomplished by the production and assembly of ECM macromolecules, by the release of growth factors for other cells and by the release of proteolytic enzymes, particularly MMPs.

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This allows fibroblasts to modulate both ECM production and destruction, as well as the response of other cells.

The current study was designed to determine if statins could modulate MMP release by human lung fibroblasts. To investigate this, we utilised two types of lung fibroblasts, the widely used human fetal lung fibroblast strain (HFL-1) [15], which allows our results to be compared with those of other investigations that have used identical or similar strains and, in addition, fibroblasts from normally appearing adult lung tissue that was removed during surgery. We have, moreover, used three statins, atorvastatin, fluvastatin and simvastatin, to assure that the effects were not specific for a single agent. In addition, to determine if the effect of statins could modulate ECM degradation in addition to MMP release and to determine if the results obtained in the conventionally used monolayer culture would be observed in other culture conditions, the effect of atorvastatin was evaluated using three-dimensional (3D) collagen gels in which collagen degradation was induced by cytokines and neutrophil elastase (NE). Finally, the signalling pathway by which atorvastatin exerts its effect on MMP release was investigated in both culture systems.

MATERIALS AND METHODS

Materials

Native type I collagen (rat tail tendon collagen) was extracted from rat tail tendons by a previously published method [16, 17]. Commercially available reagents were obtained as follows: Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were from Invitrogen Life Technologies (Grand Island, NY, USA); human NE was from Elastin Products Company, Inc. (Owensville, MO, USA); recombinant human MMP-1 and -3 Western blotting standards, recombinant human tumour necrosis factor (TNF)-α and interleukin (IL)-1α and monoclonal anti-human pro/active MMP-1 (Clone 36665) and MMP-3 (Clone 10D6) antibodies were from R&D Systems, Inc. (Minneapolis, MN, USA); horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was from Rockland (Gilbertsville, PA, USA); fluvastatin and simvastatin were from Calbiochem (San Diego, CA, USA); mevalonate, farnesyl pyrophosphate (FPP), geranylgeranyl pyrophosphate (GGPP), chloramine-T, *p*-dimethylaminobenzaldehyde and 3-(4,5-dimethylthiazolyl-2)-2,5- diphenyltetrazoliumbromide (MTT) were from Sigma (St. Louis, MO, USA).

Atorvastatin was a kind gift from Pfizer Inc. (New York, NY, USA) and was dissolved in methanol to a stock solution of 2.5 mM.

Cell culture

HFL-1 (lung, diploid, human) was obtained from the American Type Culture Collection (ATCC; #CCL-153; Rockville, MD, USA). HT1080 cells (connective tissue fibrosarcoma, diploid, human) were also purchased from ATCC (#CCL-121). HLFs were isolated from alveolar lung tissue removed at surgery for suspected malignancy that appeared to be free of cancer under a protocol approved by the Human Studies Committee of the University of Michigan (Ann Arbor, MI, USA). The cells were cultured on 100-mm tissue culture dishes (Falcon; Becton-Dickinson Labware, Lincoln Park, NJ, USA) with DMEM supplemented with 10% FCS, 100 $\mu g \cdot mL^{-1}$ penicillin, 250 $\mu g \cdot mL^{-1}$ streptomycin sulfate (penicillin-streptomycin; Invitrogen), and 2.5 $\mu g \cdot mL^{-1}$ amphotericin B (Geneva Pharmaceuticals, Inc., Dayton, NJ, USA). Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 and passaged every 3 to 5 days at a 1:4 ratio. In all experiments, HFL-1 cells between the 14th and 18th passage were used. For HLF, passages between the seventh and 11th were used. HT1080 cell culture media were used as a positive control for gelatine zymography.

To evaluate the release of MMP in monolayer culture, fibroblasts were cultured in DMEM with 10% FCS in 60-mm dishes (Falcon) at 0.5×10^6 cells for 24 h. The medium was then changed to serum-free (SF)-DMEM. After 24 h, cells were washed with phosphate-buffered saline (PBS) once and treated with statins for 1 h before stimulation with cytokines (TNF-α 5 $ng \cdot mL^{-1}$ and IL-1β 2 $ng \cdot mL^{-1}$) in SF-DMEM. After 24-h incubation, the media were harvested for gelatine zymography and Western blot analysis.

Collagen gel culture

Collagen gel cultures were conducted according to the previously published methods [17] with a cell density of 3×10^5 fibroblasts$\cdot mL^{-1}$ gel. The gels were incubated in SF-DMEM either with or without designated reagents at 37°C and 5% CO_2 for 3 days.

Hydroxyproline assay

Hydroxyproline, which is directly proportional to type I collagen content, was measured by spectrophotometry according to the previously published methods [18]. Briefly, the collagen gels were solubilised by heating in 0.1M HCl (50 μL/3 gels). Samples (20 μL) were mixed with 30 μL 3.3M NaOH and autoclaved at 120°C for 20 min. After oxidisation with 0.056M chloramine-T for 20 min, samples were reacted with Ehrlich's reagent at 65°C for 20 min. The absorbance was measured at 540 nm with BenchMark Microplate Reader and Microplate Manager III software (Bio-Rad, Hercules, CA, USA).

Western blot analysis of metalloproteinase

To assess the release of MMP-1 and -3, Western blot analysis was performed according to the previously published method [19, 20]. Briefly, the supernatants (4 mL per condition) from monolayer and 3D collagen gel cultures were precipitated with 50% (vol/vol) cold ethanol and re-suspended in 50 μL double-distilled H_2O. Because secreted MMPs were assessed, no internal standard to allow for variable dilution was used. Rather, a constant volume, 35 μL of each sample was subjected to 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel elecrophoresis (PAGE) under nonreducing conditions and then proteins were transferred to polyvinyl difluoride membranes (Bio-Rad). This then allowed comparison of the intensity of the final bands in a manner similar to immunoassay [21, 22]. After blocking with 5% nonfat milk, blots were incubated overnight at 4°C with primary antibodies (1 $\mu g \cdot mL^{-1}$ mouse anti-human MMP-1 or MMP-3 monoclonal antibody). Target proteins were detected using HRP-conjugated goat anti-mouse immunogloblin G in conjunction with an enhanced chemiluminescence detection system (Amersham Biosciences UK Limited, Little Chalfont, UK) using a Typhoon Scanner (Amersham Biosciences). Band intensity was quantified using