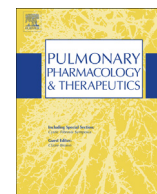


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Airway responsiveness to adenosine after a single dose of fluticasone propionate discriminates asthma from COPD

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ABSTRACT

Background: Regular treatment with inhaled corticosteroids (ICS) is known to reduce airway hyperresponsiveness (AHR) to adenosine 5'-monophosphate (AMP) in asthma even after a single dose of fluticasone propionate (FP).

Aim: To determine whether this rapid protective effect of a single dose of FP is also present in COPD.

Methods: 23 mild asthmatic and 24 COPD subjects with documented AHR to both AMP and methacholine took part in a randomized, double-blind, placebo-controlled, crossover study to measure AHR to inhaled AMP and methacholine 2 h after either 1000 µg FP or matched placebo.

Results: In subjects with asthma, 1000 µg FP in a single dose significantly attenuated the constrictor response to AMP, geometric mean (range) PC20AMP values increasing from a 19.2 (1.3–116.3) to 81.5 (9.6–1600.0) ($p < 0.001$; post-placebo vs post-FP) mg/ml. Change in the airways response to inhaled AMP after FP was well within test variability in patients with COPD, with PC20AMP values 59.6 (11.3–183.9) and 76.3 (21.0–445.3) ($p = 0.022$; post-placebo vs post-FP) mg/ml. Additionally, FP failed to significantly attenuate the bronchial response to methacholine in both asthma and COPD subjects. A change in doubling dilution, between placebo and following a single dose of FP, in AMP had a better sensitivity and specificity of 95.8% and 65.2%, compared to methacholine of 79.2% and 43.5% respectively in delineating between COPD and asthma.

Conclusion: A single dose of 1000 µg FP rapidly improves AHR to AMP in asthmatics but not in COPD subjects. This may provide a convenient way by which provocation challenge with inhaled AMP may help in discriminating asthma from COPD.

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1. Introduction

Adenosine 5'-monophosphate (AMP) is an indirect bronchostimulant with distinctive features. Given by inhalation, AMP induces dose-related bronchoconstriction in patients with asthma and, to a lesser extent, in those with COPD [1–3]. The underlying mechanism of this narrowing appears to involve the stimulation of specific mast cell surface adenosine A2b receptors with the subsequent release of mediators and contraction of airway smooth muscle [3,4].

As airway hyperresponsiveness (AHR) to AMP is more closely associated with atopy and allergic airway inflammation than AHR

to direct stimuli such as methacholine and histamine [5–7], bronchial provocation test with inhaled AMP may help to improve the diagnostic discrimination between asthma and COPD [8].

Diagnostic uncertainty can arise between COPD and asthma [9]. Indeed, not every patient with asthma presents as a 20-year-old non-smoker with atopy, dry cough, and intermittent wheezing, and not every patient with COPD presents as a 70-year-old, 110-pack-year smoker with progressive exertional dyspnoea and chronic productive cough. The diagnosis can be problematic in the middle-aged patient presenting with cough and mild exertional dyspnoea who also smokes cigarettes and spirometric data in isolation cannot be used to differentiate between the two conditions.

In contrast to what happens in COPD [10], AMP is more sensitive than methacholine in detecting changes in airway reactivity after regular treatment with inhaled corticosteroids (ICS) in asthma

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patients [11]. Moreover, it has also been shown that a single dose of inhaled fluticasone propionate (FP) is sufficient to cause a marked attenuation of the airway response to AMP in mild asthma as early as 2 h after dosing [12]. Likewise, a single dose of 2400 µg budesonide resulted in a significant 2.2-fold improvement in AHR to the mast cell dependent bronchostimulant hypertonic saline as early as 6 h in stable asthmatics [13].

We hypothesized that should the acute response to FP be absent in COPD patients, then performing a simple and convenient AMP challenge would aid in the diagnostic discrimination between COPD and asthma. Thus, the aim of the current study was to compare the acute changes in AHR to AMP after a single dose of inhaled FP in patients with asthma and COPD.

2. Methods

2.1. Subjects

Non-smoking subjects with mild asthma [14] and smokers/ex-smokers with mild to moderate COPD [15] were selected for the study. A total of forty-one (30M, 11F) consecutive patients with COPD and thirty-two (19M, 13F) with asthma were screened by bronchoprovocation testing to document AHR to both AMP and Mch. Considering that FP is known to reduce AHR, an airway sensitivity of at least PC20 ≤ 200 mg/ml and PC20 ≤ 4 mg/ml to AMP and Mch respectively had to be documented before considering COPD/asthma patients eligible for the study. Ten (5M, 5F) patients with COPD and five (3M, 2F) with asthma were unable to perform spirometry and/or provide repeatable read-outs and were excluded. Overall, twenty-four (20M, 4F) COPD and twenty-three (13M, 10F) asthma patients satisfied both PC20AMP and PC20Mch entry criteria.

Patients with COPD had no evidence of reversibility with 400 µg salbutamol, a smoking history of >10 years and an obstructive expiratory ratio. For the purpose of the study, subjects were excluded if they had a FEV₁ of less than 55% predicted for COPD patients and 70% predicted for asthmatic subjects. All asthmatics had documented evidence of atopy and positive reversibility of 12% and/or 200 ml improvement in FEV₁ with 400 µg salbutamol. Demographic and functional data of the patients are shown in Table 1. All patients were in stable clinical conditions, i.e. had no exacerbations or respiratory tract infections or had no oral steroids or anti-histamines or change to their normal ICS dose in the 6 weeks prior to enrolling onto the study. Inhaled β₂-agonists or anticholinergics were discontinued at least 24 h before the study and ICS and caffeinated beverages were restricted for at least 12 h prior

to study visits. The local ethics committee approved the study protocol and written informed consent was obtained from all patients.

2.2. Study-design

The study had a double-blind, placebo-controlled, crossover design with treatment order determined by an independent technician using a computer generated random code, and consisted of a total of four study visits 5–8 days apart. On each study visit, participants were instructed on how to use the inhalers and were asked to take two consecutive inhalations of either FP (total dose 1000 µg) or matched placebo through a Diskus® device 2 h prior to undertaking bronchial challenges with inhaled AMP or methacholine. Bronchial challenge tests were conducted randomly. Study subjects attended all visits at the same time of day (±2 h).

2.3. Bronchoprovocation testing with methacholine and AMP

AHR was evaluated by methacholine and AMP bronchial challenges as described previously [16]. In brief, methacholine (Lofarma, Milan, Italy) and AMP (Sigma Chemical Co., St Louis, USA) were dissolved in phosphate buffered saline (pH 7.4) and normal saline respectively to produce increasing doubling concentrations (0.06–16 mg/ml for methacholine; 3.125–800 mg/ml for AMP). Solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron Minineb (C.R. Bard International, Sunderland, U.K.) driven by compressed air at 8 l/min. Patients inhaled increasing doubling concentrations of agonist in five breaths from functional residual capacity to total lung capacity via a mouthpiece and FEV₁ measured at 1 and 3 min after each administration. The challenges were stopped when a decrease of 20% in FEV₁ had been achieved or when the maximum concentration of agonist had been inhaled. The bronchial responses to the inhaled agonists were expressed as the provocative concentration causing a 20% decline in FEV₁ (PC20) which was calculated by linear interpolation from the concentration–response curve constructed on a logarithmic scale.

2.4. Statistical analyses

PC20 values were logarithmically transformed to normalize their distribution and expressed as a geometric mean (range). Other baseline demographic assessments were expressed as mean (±SEM). Statistical comparisons within and between groups were carried out by paired *t*-tests and Wilcoxon Rank sum test depending on whether the data was parametrically or non-parametrically distributed, respectively. Of note, a 20% fall in FEV₁ could not be obtained in one of the participants after FP (asthmatic subject no.5) at the highest concentration of AMP administered and a conservative estimate was obtained by calculating the cumulative PC20 at the highest concentration administered. Because of this censored data, PC20 results were also compared for significance using non-parametric statistical analysis.

To compare changes in AHR after FP in asthma and COPD, the protective effect of treatment on provocation responses was calculated by comparing the difference in log PC20 after active and placebo treatments in each individual subject and expressed in terms of mean (±SD) doubling concentrations. A two-tailed *p* value of less than 0.05 was considered to indicate statistical significance. All analyses were performed with the Statistical Package for Social Science (SPSS for windows version 18.0, Chicago, IL, USA).

The specificity, sensitivity area under curve (AUC) of the methacholine and adenosine PC20 in delineating asthma and COPD was

Table 1
Demographic and functional data of study participants.

	Asthma (n = 23)	COPD (n = 24)
Sex (male/female)	(13/10)	(20/4)
Age (years)	49.9 (±6.1)	64.6 (±3.1)
FEV ₁ (% predicted)	80.4 (±3.4)	68.0 (±2.9)
FVC (% predicted)	93.0 (±3.5)	80.8 (±3.2)
FEV ₁ /FVC (%)	71.0 (±1.9)	66.3 (±1.3)
Smokers (pack/yr)	0/23	15/24 53.9 (45.3–67.5) ^a
Ex-smokers (pack/yr)	6/23 28.5 (22.2–40.2) ^a	9/24 41.8 (33.0–56.2) ^a
Salbutamol	23/23	20/24
Ipratropium bromide	0/23	6/24
Tiotropium bromide	0/23	13/24
ICS	17/23	15/24
Theophylline	0/23	5/24

Values are expressed as mean (±SEM).

ICS (inhaled corticosteroids): including beclomethasone dipropionate, budesonide, fluticasone propionate.

^a Non-parametric data expressed as median (IQR).

assessed using receiver operator characteristics (ROC) curve analysis on the change in doubling dilutions in asthma and COPD subjects between placebo and FP treatments.

3. Results

Baseline characteristics of the subjects studied are shown in Table 1. FEV₁ values at baseline were not significantly different between study days. There were no significant changes in FEV₁ after a single inhalation of 1000 µg of FP in comparison to placebo. For the COPD group, the mean (±SEM) FEV₁ values were 1.60 (±0.1) l and 1.58 (±0.1) l after placebo and FP respectively. For the asthma group, the mean (±SEM) FEV₁ values were 2.87 (±0.2) l and 2.73 (±0.3) l after placebo and FP respectively.

In the asthma group, 1000 µg FP significantly attenuated the constrictor response to AMP; geometric mean (range) PC20AMP values being 19.2 (1.3–116.3) and 81.5 (9.6–1600.0) mg/ml ($p < 0.001$) after placebo and FP treatment respectively (Table 2). In COPD patients, the airways response to AMP after FP was small and well within test variability, but significantly different from that after placebo, PC20AMP values being 59.6 (11.3–183.9) and 76.3 (21.0–445.3) mg/ml ($p = 0.022$) after placebo and FP treatment respectively (Table 2). When changes in the protective effect of inhaled FP on provocation responses were expressed as shift in doubling doses, a mean (±SD) protection of 2.08 (±0.86) and of 0.36 (±0.61) doubling doses against AMP was reported in asthmatics and COPD patients respectively (Table 2, Fig. 1A). These changes were significantly different from each other ($p < 0.001$). In the COPD study group, no statistically significant difference was found between smokers and ex-smokers in relation to acute responses to FP, their shift in doubling doses of AMP being 0.45 (±5.6) and 0.27 (±3.9) respectively ($p = 0.151$).

Inhalation of 1000 µg FP failed to significantly attenuate the bronchial response to Mch in both asthma and COPD patients. In asthma, PC20Mch values were 0.62 (0.17–2.2) and 0.77 (0.24–3.45) mg/ml after placebo and FP treatment respectively ($p = 0.083$). In

the COPD group, PC20-Mch values were 1.86 (0.22–6.06) and 2.1 (0.42–7.23) mg/ml after placebo and FP treatment respectively ($p = 0.194$). When changes in the protective effect of inhaled FP on provocation responses were expressed as shift in doubling doses, a mean (±SD) protection of 0.30 (±0.64) and of 0.20 (±0.72) doubling doses against Mch was reported in asthmatics and COPD patients respectively (Fig. 1B). These changes were not significantly different from each other ($p = 0.627$).

ROC curve analyses for change in doubling dilutions between placebo and FP for Mch and AMP respectively were performed to assess the sensitivity and specificity of these tests (Fig. 2A, B). A difference in methacholine doubling dilutions, between placebo and FP, of 0.53 demonstrated a sensitivity and specificity of 79.2% and 43.5% of delineating between COPD and asthma, respectively. Similarly a difference in AMP doubling dilutions of 1.54 demonstrated a sensitivity and specificity of 95.8% and a specificity of 65.2%, respectively. The area under the ROC curve (AUC) for difference in doubling dilutions for Mch was 0.565 (95% CI 0.398, 0.733; $p < 0.001$), which was much lower than that of AMP at 0.964 (95% CI 0.914, 1.013; $p < 0.001$).

4. Discussion

Our study set out to compare the effect of a single dose of inhaled FP (1000 µg) on AHR to inhaled AMP in subjects with asthma and COPD. FP caused a substantial reduction in the bronchoconstrictor response to AMP in subjects with asthma but not COPD, their dose–response curve being displaced to the right by 2.08 and 0.36 doubling doses, respectively. The lack of important reduction in the bronchoconstrictor response to AMP after FP in COPD patients is a major finding of this study. These discrepant responses in subjects with asthma and COPD have never been described before and may be of diagnostic utility.

The magnitude of the effects reported in the present study for asthma is similar to previous data by Ketchell et al. [12], who observed a reduction of 2.7 doubling dilutions in the airways

Table 2

Individual PC20AMP values and related shift in doubling doses (D/D) after 1000 µg FP in subjects with asthma and COPD.

Asthma patient no.	PC20AMP (mg/ml)			COPD patient no.	PC20AMP (mg/ml)		
	Placebo	FP	D/D shift		Placebo	FP	D/D shift
1	2.9	9.6	1.74	1	14.2	21.0	0.57
2	1.3	17.7	3.80	2	33.0	24.1	−0.46
3	9.4	67.2	2.83	3 ^b	56.4	31.5	−0.84
4	27.4	59.5	1.12	4 ^b	121.2	445.0	1.88
5	100.0	1600.0	4.00	5	146.5	244.9	0.74
6	28.5	66.5	1.23	6	104.2	132.2	0.34
7	8.3	46.7	2.50	7 ^b	72.0	111.8	0.63
8	64.3	410.5	2.67	8 ^b	60.1	52.0	−0.21
9	52.2	332.0	2.67	9	66.7	84.2	0.34
10	22.8	110.4	2.28	10 ^b	139.0	100.7	−0.47
11	30.7	78.9	1.36	11 ^b	178.4	245.4	0.46
12	5.6	18.3	1.71	12	42.2	60.5	0.52
13	8.5	64.4	2.92	13	183.9	207.7	0.18
14	7.7	37.5	2.28	14	144.0	107.0	−0.43
15	79.3	245.9	1.63	15	31.3	43.1	0.46
16	36.2	280.8	2.96	16	11.3	23.9	1.08
17	116.3	421.1	1.86	17	115.6	96.3	−0.26
18	49.6	195.5	1.98	18 ^b	102.9	117	0.19
19	19	52.1	1.46	19	95.7	132.7	0.47
20	5.8	18.8	1.70	20	40.8	70.6	0.79
21	21.4	33.8	0.66	21	26	45.5	0.81
22	34.1	111	1.70	22 ^b	25.5	29	0.19
23	12.9	24.2	0.91	23 ^b	16.2	36.7	1.18
				24	89.9	115.5	0.36
G. Mean (range)	19.2 (1.3–116.3)	81.5 (9.6–1600)	2.08 ^a ± 0.86		59.6 (11.3–183.9)	76.3 (21.0–445.3)	0.36 ^a ± 0.61

^a Values are expressed as mean (±SD).

^b Former smoker.

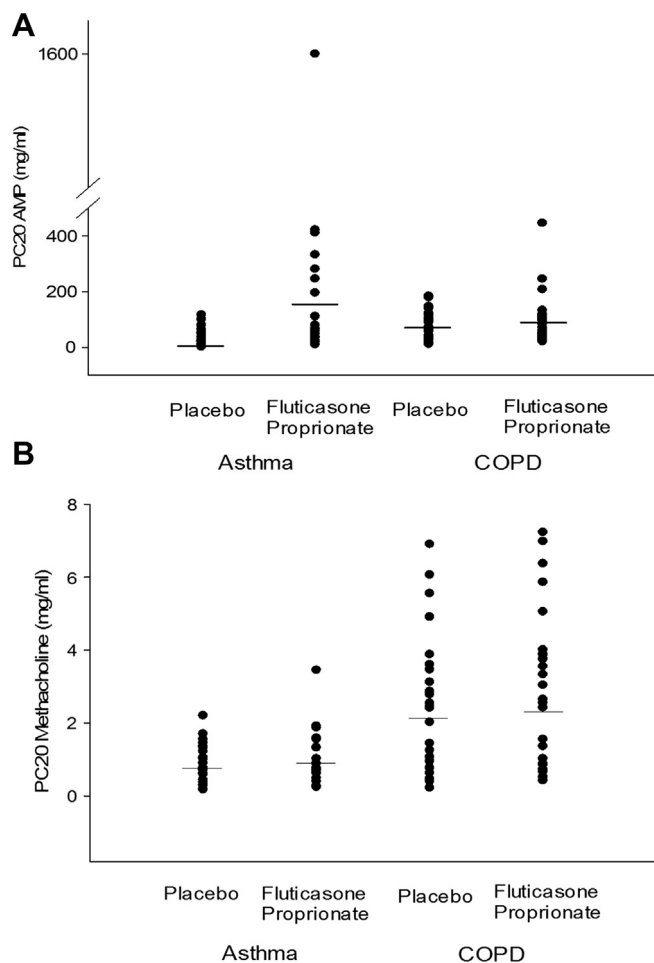


Fig. 1. Individual PC20AMP (A) and PC20 methacholine (B) values after a single dose of FP or placebo in subjects with asthma and COPD. Arithmetic means are shown as horizontal bars. Abbreviations: PC20AMP – provocation challenge 20% decline in FEV₁ after adenosine monophosphate; PC20 methacholine – provocation challenge 20% decline in FEV₁ after methacholine; FP – fluticasone propionate; COPD – chronic obstructive pulmonary disease.

response to AMP after a single dose of 1000 µg FP at 2 h. The mechanism of action for the rapid effect of ICS on AMP responses is not understood. Given that AMP-induced bronchoconstriction in asthma occurs through release of preformed and newly formed spasmogens from airway mast cells [3,4], the protection of AMP responsiveness after regular treatment with ICS has been ascribed to a reduction in mast cell number/function. Marked reductions in the number of mast cells have been observed in the bronchial mucosa of patients with asthma after regular treatment with inhaled beclomethasone dipropionate [17] or budesonide [18] probably as a result of reduction in the expression of stem cell factor (a growth factor promoting mast cell chemotaxis and differentiation) [19]. However, this is unlikely to explain how a *single* dose of FP rapidly attenuates airway response to AMP. The notion that glucocorticoids may inhibit mast cell activation via nongenomic mechanisms may provide an additional explanation. The elegant work by Zhou et al. [20] shows that inhibition of mast cell degranulation may occur as early as 10 min after budesonide in an allergic asthma model. Thus, an additional/alternative mechanism rationalizing how a *single* dose of FP may rapidly attenuate airway responsiveness to AMP may be through non-genomic mechanisms in our study.

In stark contrast to AMP, FP failed to significantly attenuate the airway response to the directly acting agonist Mch in both study

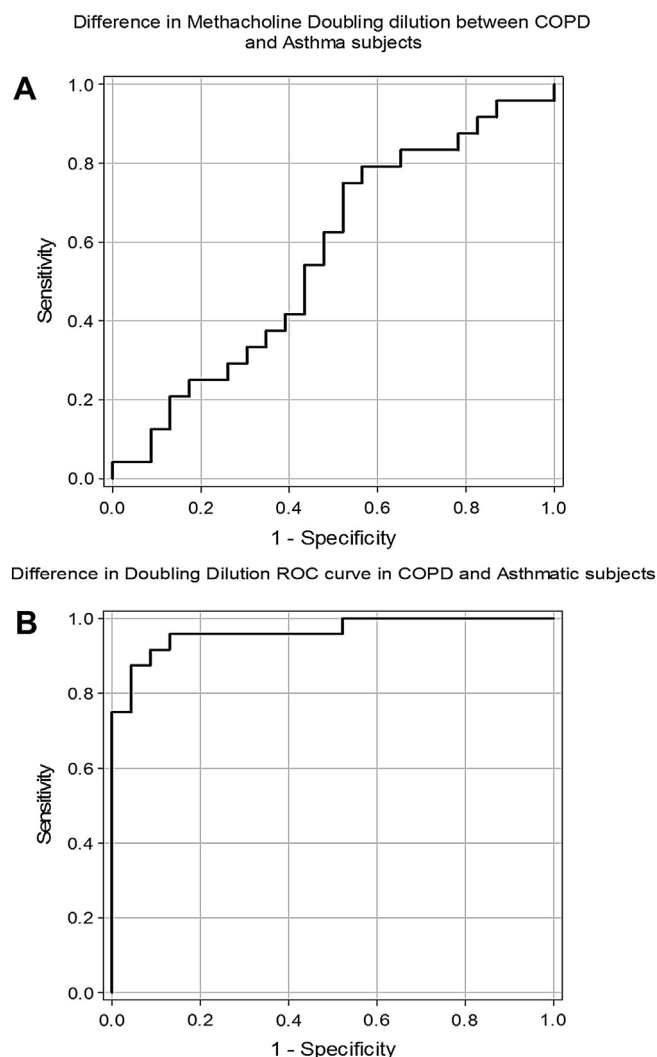


Fig. 2. Receiver operator characteristic (ROC) curves for change in doubling dilution of (A) methacholine and (B) AMP, between placebo and following a single dose of FP. Abbreviations: AMP – adenosine monophosphate; FP – fluticasone propionate.

groups. This is also in agreement with the findings of Ketchell's study [12] in which no effect was seen in the response to direct stimulus histamine in their asthmatic subjects. The lack of effect with Mch is not surprising given the modest response of directly acting agonists to regular ICS [11,21]. The explanation for the greater protective effect of ICS on AMP over Mch is not known, however may most likely be related to their different mechanism(s) of action [3,4].

Mast cells appear to play a role also in the bronchoconstrictor response to AMP of patients with COPD as indicated by clinical studies in which the anti-histamine drug terfenadine produced a significant reduction in AMP hyperresponsiveness [2]. However, the observed protective effect of terfenadine in COPD is very modest compared to asthma [16], indicating a more limited involvement of mast cells in the response of AMP in COPD. The general opinion that AHR to AMP in COPD is weaker than in allergic asthma is also supported by the present study in which basal AHR to AMP is significantly greater in asthmatics compared to COPD patients. Considering that extensive mast cell infiltration of the airway smooth muscle is a distinctive feature in asthma [22], it is likely that less responsive airways to inhaled AMP in COPD could be ascribed to a much lower number of mast cells infiltrating airway smooth muscle. Also, the nongenomic attenuation of mast cell

degranulation after a single dose of FP at 2 h is marked in the presence of a prominent mast cell phenotype (i.e. in asthma), but less evident in those conditions where mast cell infiltration of the airway smooth muscle is sparse (i.e. in COPD) [20,23]. Indeed, although a marked airways response to AMP after FP was observed in asthmatics, only a small response was shown in COPD patients.

Difference in the magnitude of the acute response reported in asthma and COPD may also be due to the different distribution in smoking status between the two study populations (all asthmatics were non-smokers; all COPD patients were smokers or former smokers). Asthma patients who smoke are known to be less sensitive to the beneficial effects of regular treatment with ICS compared to asthma patients who do not smoke (reviewed in Ref. [21]). It is not known, however, if this applies to the acute reduction in the bronchoconstrictor response to AMP after ICS. In the present study, no difference was found in the COPD study group between smokers and ex-smokers in relation to acute responses after FP. Additional evaluation of the bronchoconstrictor response to AMP after ICS in asthma patients who smoke will be necessary to establish whether smoking plays an important confounding role.

An important implication of our findings is that bronchoprovocation testing with inhaled AMP could be exploited as a useful tool to better discriminate COPD from asthma. The findings of this study clearly show that there is overlap in term of individual values of basal AHR to AMP between study groups. This could be due to the presence of smokers in the COPD study group because cigarette smoking is known to promote airway inflammation [24,25] and to affect the airway response to AMP [26]. Thus, although no diagnostic discrimination was attainable by measuring basal AHR to AMP, a single dose of 1000 µg FP elicited a significant displacement in the AMP dose–response curve in all the asthmatics, but only in one out of the 24 COPD participants. Whether this outlier reflects a case belonging to a yet undefined COPD sub-phenotype or to undiscovered asthma requires further elucidation. Of direct relevance to this isolated finding, COPD patients who have elevated numbers of eosinophils in their sputum or bronchoalveolar lavage fluid respond better to ICS [27,28] and appear to show significant improvement in the PC20AMP after ICS only when an important level of peripheral blood eosinophilia is present at baseline [10]. Unfortunately, sputum induction or blood sampling was not carried out in this study and the possibility that this COPD patient might have had airway eosinophilia cannot be tested. Nonetheless, with the exclusion of this outlier, diagnostic discrimination between asthma and COPD is clear-cut as supported by ROC curve analyses. Furthermore, the high negative predictive value of this simple procedure for COPD may allow researchers/clinicians to rapidly distinguish closely related chronic inflammatory conditions of the airways.

Although improved diagnostic discrimination between asthma and COPD is attainable by this approach, the proportion of COPD patients with an enough sensitive AMP response is a possible limitation. In the present study, about 77.5% of COPD patients had a PC20AMP of at least 200 mg/ml at screening. Hence, although diagnostic discrimination between asthma and COPD in the context of clinical research trials does not apply to all COPD patients, it is still possible in the large majority of patients.

Asthma and COPD share important similarities, but the differences are also important. Perhaps the most important difference between asthma and COPD is the nature of the inflammatory infiltrate in the airways; mainly eosinophilic infiltrate and CD4 T cells in asthma, and mainly neutrophilic infiltrate and CD8 T cells in COPD [29]. This distinction may bear relevance to the present findings because the nature of the inflammation affects the response to pharmacological agents. Effective response to ICS is well documented in eosinophilic inflammation in asthma, but not in neutrophilic inflammation of COPD [30–32]. Of note, in COPD

there are small cohorts of patients with an eosinophilic phenotype who are known to respond to regular ICS [33]. Moreover, the indirect bronchostimulant mannitol, has been reported to be associated with sputum eosinophils and to predict response to chronic dosing of ICS in COPD patients [34–36]. Although this may suggest that COPD patients with an eosinophilic phenotype may also express important reductions in the bronchoconstrictor response to AMP after ICS, there is no evidence that this occurs after acute dosing of ICS. In our study, the difference in the nature of airway inflammation in our COPD and asthma subjects may possibly account for the observed lack of attenuation in the bronchoconstrictor response to AMP after FP in our COPD patients.

A large body of evidence indicates that AHR to inhaled AMP reflects allergic airway inflammation more closely than do responses to other surrogate markers of airway inflammation. This has led to the speculation that AMP bronchial provocation may provide a dependable non-invasive tool for monitoring disease activity, and for assessing the response to anti-asthma treatments [3,4]. Here we show for the first time a rapid improvement in AMP airways responsiveness 2 h after a single dose of FP in asthma, but not in COPD. This may advocate a role for bronchial challenge with AMP to improve diagnostic discrimination between asthma and COPD and prove particularly useful in clinical research trials settings. These findings will require confirmation and validation in larger studies with unselected patients.

Competing interests

RP has received grant support from CV Therapeutics, NeuroSearch A/S, Sandoz, MSD and Boehringer-Ingelheim; has served as a speaker for CV Therapeutics, Novartis, MSD and Roche; has served as a consultant for CV Therapeutics, Duska Therapeutics, NeuroSearch A/S, Boehringer-Ingelheim and Forest Laboratories and has received payment for developing educational presentations (including service on speakers' bureaus) from MSD and Pfizer.

JBM has received lecture fees and travel grants for attending conferences from GSK.

None of the remaining authors have any competing interests to declare in relation to the topic of this manuscript.

Author contributions

LS: protocol design, recruiting of patients, interpretation of the data, writing of the ms;

VS: recruiting of patients, conduction of the study, interpretation of the data;

JBM: statistical analyses, interpretation of the data, writing of the ms;

GP: recruiting of patients, conduction of the study, interpretation of the data;

GA: interpretation of the data, writing of the ms;

MC: interpretation of the data, writing of the ms;

CF: interpretation of the data, writing of the ms;

GUDM: protocol design, interpretation of the data, writing of the ms;

RP: Principal investigator, protocol design, interpretation of the data, writing of the ms.

All authors have read and approved the final manuscript.

LS, VS and JBM contributed equally to the writing of the manuscript.

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