

Effect of methacholine challenge on cellular composition of sputum induction

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Table 1 Characteristics of subjects

Subject no.	Age (years)	Sex	PD ₂₀ (µg)	FEV ₁ * (% pred)	FEV ₁ (% pred)
1	45	M	890	113	120
2	23	F	55	95	97
3	47	F	85	92	89
4	45	M	170	110	114
5	29	M	538	97	100
6	33	F	652	99	97
7	24	F	676	112	109
8	18	F	47	89	84
9	23	F	89	107	110
10	40	F	1039	95	98
11	28	M	20	94	94
12	56	F	154	100	100
13	31	F	120	95	93
14	39	M	770	105	109
15	21	M	466	95	96
16	39	M	219	89	88
Mean	33.8			99.2	99.9
SD	11.0			7.9	10.0
Geometric mean			215		

FEV₁* = forced expiratory volume in one second performed prior to sputum induction on day without methacholine challenge.

FEV₁ = forced expiratory volume in one second performed prior to methacholine challenge.

PD₂₀ = concentration of methacholine provoking a 20% reduction in FEV₁.

Abstract

Background—Induced sputum and methacholine inhalation challenge are routinely used for the assessment of airway inflammation and airway hyperresponsiveness, respectively. This study investigates whether a methacholine challenge performed one hour before sputum induction alters the cellular and biochemical constituents of sputum.

Methods—Sixteen stable asthmatic patients with lung function within the normal range underwent two sputum inductions within one week. One hour before one of the sputum inductions a methacholine challenge was performed.

Results—There were no significant differences in total cell count, macrophages, neutrophils, eosinophils, lymphocytes, epithelial cells, ECP, and albumin between the two challenges. The repeatability of cell counts was good for all cells, ECP and albumin, but poor for total cells.

Conclusions—In patients with stable asthma a methacholine challenge carried out one hour before sputum induction does not significantly alter the cellular and biochemical constituents of sputum.

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Keywords: asthma; methacholine challenge; induced sputum

Airway inflammation and airway hyperresponsiveness are characteristics of bronchial asthma.¹ Airway hyperresponsiveness is frequently assessed by methacholine inhalation

challenge.² Airway inflammation can be assessed invasively by bronchoscopy or non-invasively by induced sputum.³⁻⁷ Because of the loose relationships between airway inflammation and airway hyperresponsiveness,⁸ these assessments may yield complementary information. In clinical studies it would be useful if airway inflammation and airway hyperresponsiveness could be measured at the same visit. To achieve this, methacholine challenge must precede sputum induction as sputum induction requires premedication with β₂ agonists.⁹

It is not known whether a methacholine challenge carried out before sputum induction alters the sputum cell count. This study compares the total and differential cell counts as well as the biochemical characteristics (ECP, albumin) of induced sputum obtained with or without a prior methacholine inhalation challenge.

Methods

SUBJECTS

Sixteen non-smoking subjects with bronchial asthma, defined as a clinical history of intermittent wheeze, cough, chest tightness, or dyspnoea, participated in the study (table 1). Subjects were taking only inhaled short acting β₂ agonists on an as needed basis for their asthma.

At the time of the study all subjects were in a stable clinical condition as demonstrated by the low daily variability (<15%) of peak flow measurements during the two weeks before the study and by the low variability in forced expiratory volume in one second (FEV₁) performed in the two days of the study (table 1). All subjects had lung function within the normal range and methacholine airway responsiveness with the dose required to provoke a fall in FEV₁ of 20% or more (PD₂₀FEV₁) of <1600 µg.¹⁰ Each subject attended the laboratory on two occasions within one week at least 48 hours apart. On one occasion, at random, sputum induction was performed one hour after methacholine challenge. The sputum induction was performed at the same time of the day on the two occasions.

The study was approved by the ethics committee of Fondazione Maugeri and all subjects gave written informed consent.

METHACHOLINE CHALLENGE

Methacholine (Sigma Chemicals Co, St Louis, Missouri, USA) was dissolved in distilled water and delivered by an ampoule dosimeter device (Mefar, Brescia, Italy) driven by compressed air at a pressure of 1.5 kg/m² with one second actuations and five second intervals between breaths. Aerosols were inhaled during quiet

tidal breathing. After inhalation of isotonic saline as a control, doubling doses of methacholine were inhaled from 20 to 1600 µg. A three minute interval was allowed before each dose increment. FEV₁ was measured one minute after each dose and the best of three acceptable measurements was retained to create dose-response curves. The non-cumulative PD₂₀FEV₁ values were calculated by interpolation between two adjacent points of the log dose-response curves.

SPUTUM INDUCTION

Inhalation procedure

After baseline FEV₁ and forced vital capacity (FVC) measurements were taken, salbutamol was given by inhalation (200 µg by MDI) and subjects inhaled hypertonic (4.5%) saline nebulised for periods of progressively increasing length (1, 2, 4, 8, 16 minutes). FEV₁ was again measured one minute after each inhalation period. Saline solutions were nebulised by an ultrasonic nebuliser (DeVilbiss 65, DeVilbiss Corp, Somerset, Pennsylvania, USA). On the methacholine challenge day salbutamol (200 µg by MDI) was given at the end of the challenge to hasten the recovery of lung function.

Sputum processing

The collected sputum samples were examined within two hours. Selected portions of the sputum sample originating from the lower respiratory tract were chosen using an inverted microscope and weighed. Dithiothreitol (DTT, Sputolysin, Calbiochem Corp, San Diego, California, USA), freshly prepared in a dilution of one in 10 with distilled water, was added in a volume (in litres) equal to four times the weight of the sputum portion (in mg). Selected sputum was placed in a shaking water bath at 37°C for 20 minutes to ensure complete homogenisation. It was further diluted with phosphate buffered saline in a volume equal to the sputum plus DTT. The suspension was filtered through gauze to remove mucus and was centrifuged at 1000g for five minutes. The supernatant was aspirated and frozen at -70°C for later ECP (µg/ml) and albumin (µg/ml)

analysis by radioimmunoassay (RIA; Kaby Pharmacia Diagnostic AB, Uppsala, Sweden). The cell pellet was resuspended in a volume of PBS equal to that of the sputum plus DTT and PBS as above. Total cell count (TCC) and viability (Trypan blue exclusion method) were determined using a Burke's chamber haemocytometer. The cell suspension was placed in a Shandon 3 cyto centrifuge (Shandon Southern Instruments, Sewickley, Pennsylvania, USA) and cytopins were prepared at 450 rpm for six minutes. Cytospin slides were fixed in methanol and stained with May-Grunwald Giemsa for an overall differential cell count of 500 nucleated non-squamous cells. Only samples with cell viability of >50% and squamous cell contamination of <20% were considered adequate. All subjects were able to produce an adequate sample on the two occasions. All sputum counts and measurements were performed blind to the clinical details.

STATISTICAL ANALYSIS

Descriptive statistics were used to summarise the clinical and demographic characteristics of the subjects. Data were reported as the arithmetic mean and standard deviation. PD₂₀ data were log transformed and reported as geometric means. Wilcoxon signed rank tests were used to compare cell counts and biochemical markers of the two sputum samples. The repeatability of measurements was examined by the reliability coefficient (intraclass correlation coefficient = R) as the proportion of the variance in the measures due to the true variance between subjects. Data are presented as median and interquartile range. A value of p<0.05 was considered statistically significant.

Results

Total and differential cell counts with and without methacholine challenge are shown in table 2. There were no significant differences in total cell count, percentage or absolute numbers of sputum macrophages, neutrophils, eosinophils, lymphocytes, and epithelial cells with and without methacholine challenge.

Table 2 Induced sputum total and differential cell count and fluid analysis (ECP and albumin) when performed (A) without and (B) with methacholine challenge

Patient no.	TCC (A)	TCC (B)	Mac (A)	Mac (B)	Neu (A)	Neu (B)	Eos (A)	Eos (B)	Lym (A)	Lym (B)	Epi (A)	Epi (B)	ECP (A)	ECP (B)	Alb (A)	Alb (B)
1	800	1600	9.8	7.6	76	79.4	13.8	12.8	0.4	0	0	0.2			664	424
2	80	384	15	22.4	83.5	76.4	1.5	0.4	0	0.4	0	0.2				
3	1680	880	64.8	33.6	12.4	16.4	18	42	1.2	0.4	3.6	7.6				
4	880	1840	22.6	18.4	76.2	80.2	1.2	1	0	0.2	0	0.2	624	142	232	136
5	1280	2240	55.6	31.8	38.4	64.4	5.6	3.6	0.4	0	0	0.2	960	2080	1280	1776
6	440	800	58.4	45.2	39.2	51	0.2	0.4	2	3.4	0.2	0				
7	320	128	57.6	49	40.4	49.6	0	0	1	0	1	1.4	40	33	120	40
8	96	360	67.2	61.4	11.2	16.2	20.8	21	0	0	0.8	1.4				
9	160	280	81.8	80.8	15.6	14.8	2.4	3.2	0.2	0.6	0	0.6	480	608	120	320
10	480	96	40.4	57.4	59.2	42	0	0.2	0	0	0.4	0.4	520	96	552	384
11	640	336	55.2	50.4	41.4	45	3	4.2	0	0.4	0.4	0	976	1104	320	728
12	480	240	62.4	51.4	28.8	42	7.2	5.6	1.6	0.8	0	0.2	1200	1520	224	632
13	480	336	77.2	73	22	26.6	0.4	0	0.4	0.2	0	0.2				
14	336	880	29.4	26.4	69.2	72.8	0.8	0.4	0	0	0.6	0.4	600	416	152	216
15	800	560	62.6	68	27.8	25.4	8.4	6.4	0	0	1.2	0.2				
16	312	536	42.6	44.2	5.6	3.6	51.6	52	0.2	0	0	0.2				
Median	480	460	56.6	47.1	38.8	43.5	2.7	3.4	0.2	0.1	0.1	0.2	612	512	232	384
Range	80-1680	96-2240	9.8-81.8	7.6-80.8	5.6-83.5	3.6-80.2	0-51.6	0-52	0-1.6	0-3.4	0-3.6	0-7.6	40-1200	33-2080	120-1280	40-1776
Wilcoxon	p = 0.32		p = 0.08		p = 0.06		p = 0.49		p = 0.69		p = 0.22		p = 1.0		p = 0.37	
R	0.56		0.83		0.93		0.91		0.71		0.73		0.66		0.81	

TCC = total cell count (no. cells/ml × 10⁴); Mac = macrophages (%); Neu = neutrophils (%); Eos = eosinophils (%); Lym = lymphocytes (%); Epi = epithelial cells (%); ECP = µg/ml; albumin = µg/ml; R = coefficient of reliability.

There was no significant difference between sputum cell viability with (median 79%, range 59–88) and without (median 80%, range 76–90) methacholine challenge. Again, no significant differences were noted in sputum ECP and albumin levels. The repeatability (R) of cell counts was good for all cells, ECP and albumin, but poor for total cells.

Discussion

This study in asthmatic patients shows that a methacholine challenge carried out one hour before induction of sputum does not significantly alter the cellular and biochemical constituents of the sputum.

The effects of methacholine challenge on bronchoalveolar lavage (BAL) fluid cell count have been extensively evaluated in previous studies. Lam *et al* showed that a methacholine challenge performed 10 minutes before bronchoscopy did not alter significantly the BAL fluid cell counts.¹¹ Beasley *et al* found that the numbers of nucleated cells in BAL fluid were increased 18 hours after methacholine challenge in asthmatic subjects. However, the percentages of each inflammatory cell type, such as lymphocytes, eosinophils, neutrophils and macrophages, were unchanged.¹² In contrast, Soderberg *et al* found that neither cell count nor albumin concentration were changed in BAL fluid 24 hours after methacholine challenge.¹³ Although the results of BAL fluid and sputum studies cannot be directly compared, the results of the present study confirm that methacholine challenge does not alter significantly the assessment of airway inflammation. We believe our

results may be helpful for both clinical purposes and for designing clinical trials in which information on airway inflammation and airway hyperresponsiveness is sought.

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