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Effects of inhalation of *N*-formyl-methionyl-leucyl-phenylalanine in the well elderly and in patients with chronic bronchitis

M J Peters, A B X Breslin, N Berend

Abstract

Background **Inhalation of the bacterial peptide *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) produces bronchoconstriction in normal subjects. FMLP thus has a putative role as a mediator of bronchoconstriction associated with bacterial bronchial infection.**

Methods **The effects of FMLP inhalation were examined in ten subjects with a history of chronic bronchitis and ten age matched control subjects. Each subject inhaled FMLP doses from 0·025 to 0·8 μmol to determine the provocative dose of FMLP causing a 20% fall in FEV_1 (PD_{20}FMLP). FEV_1 was recorded every five minutes after the final FMLP inhalation until it had returned to 95% of baseline FEV_1 or 60 minutes had elapsed. The time to return to 95% baseline FEV_1 was recorded or extrapolated from the recovery curve as an index of rapidity of recovery. Total and differential white cell counts were performed on each subject at baseline and five and 15 minutes after the final FMLP inhalation.**

Results **The geometric mean PD_{20} FMLP in the patients with chronic bronchitis was 0·06 μmol (95% confidence interval 0·015–0·26), which was significantly lower than that in the control subjects (0·21 μmol (0·02–1·9)). PD_{20} FMLP in the patients with chronic bronchitis but not age matched controls ($p = 0·35$) was lower than that found previously in young normal subjects (0·35 μmol (0·07–1·8). The return to 95% baseline FEV_1 occurred after 86(10) minutes in subjects with chronic bronchitis and in 81(23) minutes in their age matched controls, in both cases being much slower than that seen in young subjects (29(9) minutes).**

Conclusion **Patients with chronic bronchitis may be especially susceptible to formyl peptides elaborated by bacteria during bacterial bronchial infection.**

Department of Thoracic Medicine, Concord Hospital
M J Peters
A B X Breslin

Department of Medicine, Sydney University, Sydney, Australia
N Berend

Reprint requests to:
Dr M J Peters,
Department of Thoracic Medicine, National Heart and Lung Institute, London SW3 6LY, UK

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Bacterial bronchial infection is the commonest cause of acute respiratory failure in subjects with chronic airflow limitation.[1 2] Mucosal oedema, mucus hypersecretion and mucus plugs may all contribute to the increase in airway resistance. Release of specific mediators by bacteria may cause histamine release[3] or enhanced α adrenergic responsiveness.[4 5] Formyl peptides such as *N*-formyl-ethionyl-leucyl-phenylalanine (FMLP) are products of protein synthesis[6 7] in proliferating bacterial[8] and eukaryotic mitochondria.[9] FMLP contracts human bronchial smooth muscle in vitro[10] and inhaled FMLP is a potent bronchoconstrictor in normal subjects.[11] When nebulised intermittently for two hours in rabbits, FMLP produces marked airway inflammation.[12] FMLP may therefore be an important mediator of both bronchoconstriction and airway inflammation associated with bacterial bronchial infection.

Patients with chronic bronchitis, in contrast to subjects with a similar smoking history but without sputum production, have mucosal and periglandular inflammation in large airways with neutrophil infiltration.[13] Although FMLP appears to have a direct contractile effect on human bronchial smooth muscle in vitro,[10] both neutrophils and macrophages release mediators in response to FMLP and these may contribute to bronchoconstriction in vivo.[14] FMLP induced bronchoconstriction in vivo in rabbits is partially dependent on neutrophils.[15] Increased local protease activity associated with airway inflammation may alter FMLP degradation and bioactivity.

Thus there may be differences in the response to FMLP between normal young subjects and older subjects with chronic bronchitis, who are most susceptible to the development of respiratory failure during bacterial bronchial infection. The purpose of this study was to determine the effects of inhaled FMLP on subjects with chronic bronchitis and to compare changes in airway function and haematological changes after FMLP with age matched controls.

Methods

Ten subjects with a history of cough and sputum production consistent with the diagnosis of chronic bronchitis and ten age matched controls without chronic bronchitis were selected for the study. Subjects were recruited from patients referred for resection of lung cancer, except for three control subjects (one asymptomatic, one with atypical chest pain and one waiting for hip surgery). The control group consisted of six former smokers without chronic bronchitis and four subjects who had never smoked. Subjects in both groups were

aged between 60 and 70 years and none had a history of asthma or recent respiratory infection. The presence or absence of chronic bronchitis and smoking history were determined by clinical interview before lung function testing and bronchial provocation. No subject was taking inhaled or oral corticosteroid or theophylline.

Each subject performed a histamine challenge using the method of Yan.[16] The provocative dose of histamine needed to cause a 20% fall in FEV_1 (PD_{20} histamine) was always greater than 2 μmol. The histamine challenge was performed on a separate day before the FMLP challenge. Subjects in the chronic bronchitis group, still smoking at the time of entry, were not challenged with FMLP or histamine until they had ceased smoking for seven days. The protocol was approved by the institutional ethics committee of Concord Hospital. All subjects gave informed consent for participation.

FMLP PROVOCATION

FMLP (Sigma Chemicals) at a concentration of 5 mmol/l was dissolved in 50% dimethyl sulphoxide (DMSO)/saline and diluted with saline to produce doubling dilutions down to 150 μmol/l. Five breaths of each concentration of solution were taken from a de Vilbiss 646 nebuliser powered by air at a flow rate of 8 l/min and pressure of 138 kPa, a French-Rosenthal dosimeter being used with duration of nebulisation 0·6 seconds. FEV_1 performed after inhalation of 25% DMSO/saline was used as control. The range of FMLP doses inhaled was 0·025–0·8 μmol. FEV_1 was recorded three minutes after each inhalation, the endpoint of challenge being a 20% fall in FEV_1. PD_{20} FMLP was determined by linear interpolation of the log-dose response curve. For statistical purposes subjects with a 20% fall in FEV_1 after the initial dose of FMLP were assigned a PD_{20} FMLP of 0·025 μmol; those who had a fall in FEV_1 of less than 20% after the largest dose of FMLP were assigned a PD_{20} FMLP of 0·8 μmol.

RECOVERY

FEV_1 was recorded at five minute intervals after the final dose of FMLP for 60 minutes or until FEV_1 had returned to 95% of the baseline value. The rate of recovery from induced bronchoconstriction was assessed only in subjects who sustained a 20% or greater FEV_1 fall in response to FMLP. The time to return to 95% of baseline FEV_1 was determined by interpolation or linear extrapolation of the FEV_1-time curve.

HISTAMINE RESPONSIVENESS

When assessment of recovery ceased (at 60 minutes or return to 95% baseline FEV_1), a further histamine provocation test was performed.

CIRCULATING LEUCOCYTES

Venous blood was taken at baseline and at five and 15 minutes after the final FMLP dose to determine the effect of FMLP inhalation on circulating leucocyte count. The total white cell count was done on a Coulter counter and differential leucocyte count on 100 cells after staining with May-Grünwald-Giemsa.

EFFECT OF THE DMSO DILUENT

Subjects with chronic bronchitis took six inhalations, each of five breaths of 25% DMSO/saline on a second day. FEV_1 was recorded two minutes after each inhalation. Histamine challenge was performed and blood taken at the same time as those on the FMLP day. The order in which DMSO and FMLP challenge was performed was randomised.

DATA FROM YOUNG SUBJECTS

FMLP responsiveness and recovery from FMLP induced bronchoconstriction in young subjects was taken from results previously published.[11] FMLP provocation and determination of recovery time was determined in identical fashion for these subjects except that the highest FMLP dose administered was 1·6 μmol rather than 0·8 μmol.

ANALYSIS

Group comparisons were made between the subjects with chronic bronchitis, the age matched controls and previous data from normal young subjects.[11] In examining values for PD_{20} FMLP from normal young subjects, those which were greater than 0·8 μmol were censored to the maximum dose given in this study (0·8 μmol). Log PD_{20} FMLP and time to return to baseline were compared by the Wilcoxon rank sum test.

Comparison of PD_{20} histamine after FMLP and DMSO was made on log transformed data by the Wilcoxon test for paired differences. White cell and differential leucocyte counts were compared by using the Student's t test. Correlations were determined by non-parametric regression. Data are presented as mean (SE) or geometric mean (95% confidence limits) as appropriate.

Results

The mean age of the patients with chronic bronchitis was 66 years (range 62–70 years), compared with 65 years (range 63–69 years) in the elderly control subjects. The mean age of the young control subjects was 35 years (range 28–42 years). The mean (SE) baseline FEV_1 value in the patients with chronic bronchitis was 72(2·1)% of predicted, and in the elderly controls 82(5·3)% of predicted ($p > 0·1$). Mean (SE) baseline FEV_1 in the young control subjects was 94(4·1)% of predicted, significantly greater than that in patients with chronic bronchitis but not elderly controls. Patients with chronic bronchitis had smoked a mean total of 59(3) pack years. The six former smokers in the age matched control group had smoked a mean of 55 pack years with a mean non-smoking interval of seven years (range 2–15 years). Baseline histamine responsiveness was similar in the two older groups. Geometric mean PD_{20} histamine in the patients with chronic bronchitis was 6·9 μmol (4·7–10·2

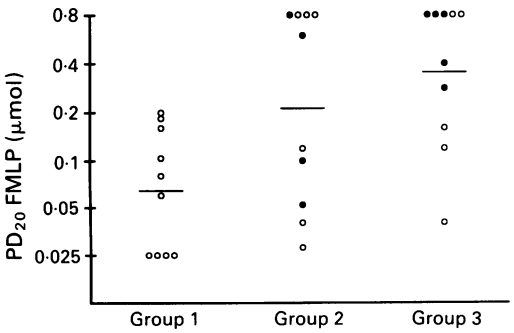

Figure 1 PD_{20}FMLP illustrated for subjects with chronic bronchitis (group 1), age matched control subjects (group 2) and young healthy subjects (group 3). Horizontal lines indicate geometric mean PD_{20}FMLP in each group. Closed circles in groups 2 and 3 indicate lifelong non-smokers. PD_{20}FMLP is significantly lower in the patients with chronic bronchitis than in both other groups. There is no difference between the older and younger control subjects.

μmol) compared with 6·7 μmol (range 3·9–11·6 μmol) in the control group.

EFFECT OF FMLP

No subject in either older group exhibited facial flushing in response to FMLP. Geometric mean PD_{20} FMLP in the patients with chronic bronchitis was 0·06 μmol (0·015–0·26 μmol) compared with 0·21 μmol (0·02–1·9 μmol) in the age matched control subjects and 0·35 μmol (0·07–1·8 μmol) in the young normal subjects (figure 1 and table). The difference between PD_{20} FMLP in patients with chronic bronchitis and young normal subjects as 2·5 doubling dilutions (95% confidence interval of the difference 1·7–3·2); that between patients with chronic bronchitis and their age matched controls was 1·7 doubling dilutions (0·29–3·2). There was no significant difference between the young subjects and the older control group (95% confidence interval of the difference: −0·5–1·9 doubling dilutions). In the elderly control group there was no difference in PD_{20} FMLP between former smokers and those who had never smoked. There was no correlation in either group between log PD_{20} FMLP and baseline FEV_1 or log PD_{20} histamine.

RECOVERY

All of the patients with chronic bronchitis and six of the older control subjects (three ex-smokers and three who had never smoked) had a 20% or greater fall in FEV_1 in response to FMLP. In the patients with chronic bronchitis, mean (SEM) time to return to 95% baseline FEV_1 was 86(10) minutes which was not significantly different from the 81(23) minutes observed for the older control subjects (figure 2). The duration of bronchoconstriction was significantly longer in both older groups than recovery in healthy younger subjects, in whom FEV_1 returned to 95% of baseline in 29(9) minutes (table).

EFFECT OF FMLP INHALATION ON HISTAMINE RESPONSIVENESS

Repeat histamine challenge in one subject with chronic bronchitis was not possible after FMLP because FEV_1 was too low 60 minutes after the final FMLP dose. Geometric mean PD_{20} histamine in the nine subjects evaluable was 6·9 μmol (4·7–10·2 μmol) at baseline, 4·6 μmol (1·3–18·0 μmol) after FMLP inhalation and 5·7 μmol (3·0–10·9 μmol) after DMSO. Neither FMLP nor DMSO produced a significant change in PD_{20} histamine. In the age matched control subjects PD_{20} histamine after FMLP inhalation was 4·0 μmol (0·57–27·9 μmol), a value that also did not differ from baseline.

EFFECT OF FMLP ON CIRCULATING LEUCOCYTES

The only change in circulating leucocyte count to achieve significance was a fall in monocyte count 15 minutes after the final FMLP dose in the patients with chronic bronchitis (figure 3). Two subjects in this group and five in the control group showed a fluctuation in either direction of greater than 25% of baseline

Characteristics of subjects for each group

Patient No	*Sex*	*Age (years)*	*FEV_1 (% pred)*	*Smoking (pack years)*	*PD_{20} FMLP (μmol)*	*Duration of bronchoconstriction (min)*
Group 1: Patients with chronic bronchitis						
1	M	69	72	65	0·16	76
2	M	64	76	40	0·18	64
3	M	62	63	50	0·10	81
4	M	69	73	60	0·20	63
5	M	69	75	60	0·025	150
6	M	70	57	60	0·025	71
7	M	64	62	60	0·06	143
8	F	64	83	50	0·08	78
9	M	64	76	60	0·025	72
10	F	66	84	80	0·025	63
Mean		66	72		0·06	86
Group 2: Elderly control subjects						
1	M	63	69	50	>0·8	
2	M	65	67	45	>0·8	
3	F	63	69	50	0·04	134
4	M	65	59	60	0·03	168
5	M	64	103	55	>0·8	
6	M	64	74	70	0·12	45
7	M	65	98	0	0·60	41
8	M	66	106	0	>0·8	
9	F	64	92	0	0·10	50
10	M	69	85	0	0·05	49
Mean		65	82		0·21	81
Group 3: Young subjects						
1	M	38	105	0	1·2	9
2	M	40	97	0	0·4	90
3	F	29	84	0	0·28	15
4	F	30	106	0	0·8	24
5	M	40	77	0	1·6	21
6	F	29	111	5	0·12	18
7	F	30	82	9	>1·6	
8	M	31	100	10	0·16	21
9	M	42	76	22	0·04	33
10	M	40	97	20	>1·6	
Mean		35	94		0·35	29

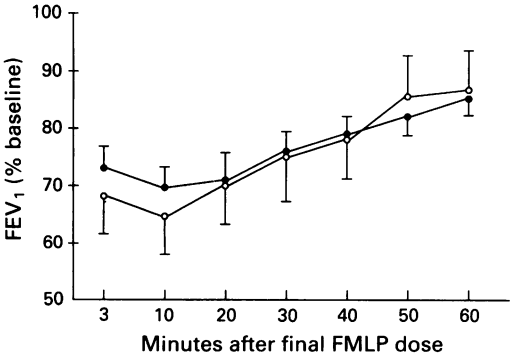

Figure 2 Recovery from FMLP induced bronchoconstriction in all 10 bronchitic subjects (closed circles) and the six age matched control subjects (open circles) who had a 20% FEV_1 fall in response to FMLP. Both groups recover more slowly than young subjects with a similar initial fall in FEV_1.

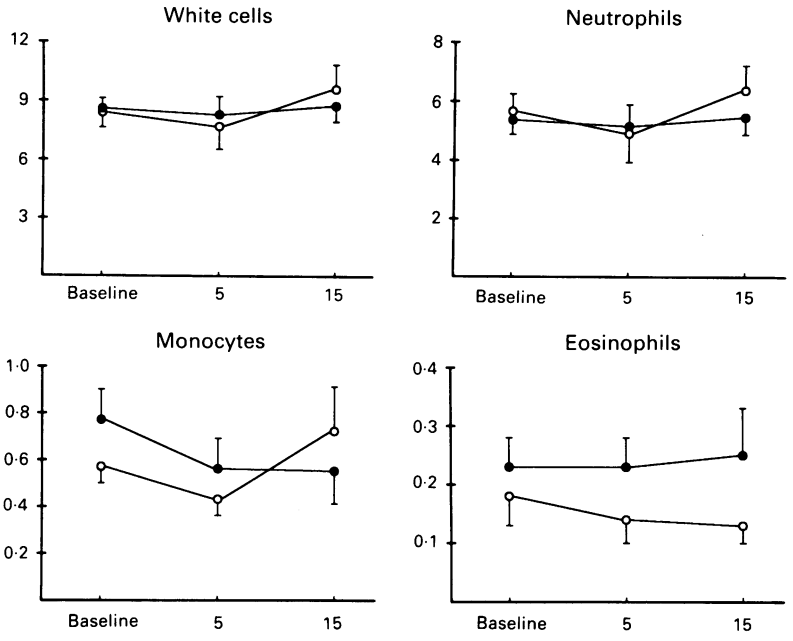

Figure 3 Mean (SEM) peripheral blood cell counts ($\times 10^9/l$) in patients with chronic bronchits (closed circles) and well elderly control subjects (open circles) at baseline and five and 15 minutes after the final FMLP inhalation.

neutrophil count. Change in neutrophil count did not correlate with total FMLP dose.

EFFECTS OF THE DMSO DILUENT

Mean (SEM) FEV_1 after inhalation of DMSO/saline alone in the subjects with chronic bronchitis was 97·6(1·0)% of the baseline value, the greatest fall being 5·4%. PD_{20} histamine after DMSO remained within one doubling dilution of the baseline value in all subjects. There was no change in total or differential white cell count.

Discussion

Bacterial bronchial infection is the most common precipitant of acute respiratory failure in subjects with chronic airflow limitation[1 2] and a major cause of morbidity and mortality. We found that subjects with chronic bronchitis bronchoconstricted to lower doses of inhaled FMLP and recovery from FMLP induced bronchoconstriction was delayed in the older subjects irrespective of the presence of chronic bronchitis.

There was no difference in PD_{20} FMLP, recovery from bronchoconstriction or in the haematological effect of FMLP inhalation between the ex-smokers and those who had never smoked in the control group, so we feel that they represent a valid control group. The differences in FMLP responses between the bronchitic subjects and the ex-smoking controls occurred despite a close match for total smoking history. This implies that excess sputum production predicts greater sensitivity to FMLP inhalation.

Small airways of smokers with airflow obstruction have thickened walls and smaller luminal diameters than those of control subjects.[17] As a result, the reduction in airflow would be expected to be greater in patients with chronic bronchitis for an equivalent degree of smooth muscle contraction. If this was the sole explanation for the enhanced response to FMLP, a similarly increased response to histamine inhalation should have been present, yet PD_{20} histamine was similar in the two older groups of subjects. This suggests that some other feature of chronic bronchitis leads to enhanced FMLP responsiveness.

Subjects with chronic bronchitis have airway inflammation with an excess of neutrophils in the mucosa.[13] Tissue localised neutrophils[18 19] and macrophages,[20] which mediate FMLP induced bronchoconstriction in the guinea pig,[21] may be primed in the presence of inflammation to produce an enhanced response to stimulation by FMLP. Alternatively, mucosal inflammation, which increases permeability to formyl peptides,[22] may permit greater access for FMLP across bronchial epithelium to receptors within the submucosa, vasculature and bronchial smooth muscle. Airway inflammation in chronic bronchitis could modify the bronchoconstrictor response to FMLP by either mechanism.

Recovery from FMLP induced bronchoconstriction was much slower in both older groups of subjects than in the younger subjects studied earlier. The observed difference was not dose related, as the young subjects, by virtue of their higher PD_{20} FMLP values, had inhaled more FMLP than either older group. Nor was it a function of a greater initial response to FMLP, as only subjects with a 20% or greater fall in FEV_1 were compared. It is not a non-specific effect of age as older subjects recover from histamine induced bronchoconstriction at a rate similar to that of younger subjects (Peters, unpublished observations).

One explanation for this prolonged duration of effect would be that metabolism of FMLP slows with age. In view of its distribution in the lung,[23] FMLP hydrolysis in bronchial mucosa is likely to be via the enzyme neutral endopeptidase as in neutrophils.[24] Inhibition of neutral endopeptidase enhances the effect of FMLP on cholinergic neurotransmission in rabbit airway in vitro[25] though we found a pronounced reduction in the bronchoconstrictor response to FMLP inhalation after inhibition of neutral endopeptidase in vivo.[26] Neutral endopeptidase activity does not decline with age and, if anything, serum activity is increased modestly in subjects with chronic airflow limitation.[27] The prolonged response to FMLP in the older subjects cannot be explained by changes in neutral endopeptidase.

The neutrophil dependent oedema seen in rabbit skin after FMLP injection is markedly accentuated by vasodilator pretreatment and has a halflife of 40 minutes.[28] Persistence of airway oedema could explain the prolonged effect of FMLP in older subjects but whether clearance of oedema slows with age or bronchoconstriction is related more closely to airway oedema in this group is unknown. Leucocyte depletion[15] and anticholinergic agents[23] inhibit FMLP induced bronchoconstriction but there is no evidence of differences in the cholinergic or leucocyte response to FMLP in this age group to account for the slower recovery.

FMLP inhalation did not produce changes in neutrophil or monocyte counts in venous

blood in either group of the magnitude that we had seen in normal subjects.[29] There was also a total absence of facial flushing in both older groups after FMLP inhalation whereas flushing is seen in over 75% of young subjects,[11 29 30] always occurring after the first FMLP dose and always associated with marked neutropenia.[29] Transient neutropenia after FMLP inhalation probably reflects synchronous activation of circulating neutrophils and temporary sequestration in the microcirculation. We have usually given a single inhalation of 0·4 μmol FMLP to young subjects after finding variability in the neutrophil responses to incremental doses of FMLP (0·1, 0·5, 1 μmol).[31] Nevertheless, all young normal subjects inhaling the incremental doses of FMLP had either a transient neutropenia or neutrophilia or both; in this study even the five older control subjects who had a dose of FMLP equivalent to that given to young normal subjects had a minimal neutrophil leucocytosis following FMLP.

The leucocyte response to FMLP may require access of FMLP to the circulation. Neutrophils from well older subjects have higher resting intracellular calcium concentrations and the response to a variety of stimuli including FMLP is slightly reduced.[32 33] A final possibility is that the neutrophils of older subjects, behave in a similar way to those of younger subjects but less in synchrony so that flushing and profound neutropenia which may require synchronous neutrophil activation adherence are not seen. Although the neutropenic response was absent in the older subjects, bronchoconstriction was similar in the older control subjects to that in the younger normal subjects and was increased in subjects with chronic bronchitis. The role that leucocytes play in FMLP induced bronchoconstriction is clearly not linked directly to the development of neutropenia in either age group.

In this study, subjects with chronic bronchitis showed greater sensitivity to the effects of inhaled FMLP and older subjects showed delayed recovery from FMLP induced bronchoconstriction in comparison with young subjects. The combination of disease related increased sensitivity and age related slowing of recovery would place an older patient with chronic bronchitis at special risk of the effects of FMLP and related formyl peptides produced by bacteria during bacterial bronchial infection.

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Notes