Effect of inhaled formyl-methionyl-leucyl-phenylalanine on airway function

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ABSTRACT   Formyl-methionyl-leucyl-phenylalanine (FMLP), a synthetic, acylated tripeptide analogous to bacterial chemotactic factors, has been shown to cause bronchoconstriction in guinea pig, rabbit, and human airways in vitro. To determine whether FMLP causes bronchoconstriction in man in vivo, a preliminary study was undertaken in which five non-smokers (mean age 35 years, FEV_1 94% (SEM 5%) predicted) and five smokers (mean age 34 years, FEV_1 93% (6%) predicted) inhaled aerosols of FMLP. None of the subjects showed airway hyperresponsiveness to histamine (the provocative concentrations of histamine causing a fall of $\geqslant$ 20% in FEV_1 (PC_{20}) were over 8 mg/ml). FMLP dissolved in 50% dimethylsulphoxide and 50% saline in concentrations of 0, 0·06, 0·12, 0·25, 0·5, 1·0, 2·0, and 4·0 mg/ml was administered to the subjects by means of a French-Rosenthal dosimeter, FEV_1 being recorded after inhalation of each concentration. Dose dependent falls in FEV_1 occurred in five non-smokers (geometric mean 1·76, 95% confidence limits 0·87–3·53 mg/ml) and three smokers (0·23, 0·07–0·78 mg/ml), with two smokers not responding by 20% to the highest concentration of FMLP. On a separate day the FMLP dose-response curves were repeated after nebulisation of 500 μg of ipratropium bromide. The PC_{20} FMLP in the responders more than doubled. In six additional normal subjects a histamine inhalation test was performed before and four and 24 hours after inhalation of FMLP. All subjects remained unresponsive to histamine. These results show that FMLP is a potent bronchoconstrictor in some non-asthmatic individuals in vivo and this may be important in bronchoconstriction related to infection in patients with chronic obstructive lung disease.

Bacteria elaborate chemotactic factors for leucocytes.[1-3] These factors have been isolated and characterised as small molecular weight peptides.[4-5] In subsequent studies of the structure-activity relations of synthetic chemotactic peptides[6] the acylated tripeptide formyl-methionyl-leucyl-phenylalanine (FMLP) was shown to be the most active chemotactic agent. With an ED_{50} for rabbit neutrophil chemotaxis of 70 pmol/l, FMLP is one of the most potent chemotaxins known. The well defined chemical structure of the ligand has permitted detailed studies of the nature of the receptor that is present on macrophages and

neutrophil polymorphonuclear leucocytes.[7-10] FMLP stimulates many functions of these cells, including granule enzyme secretion,[11-12] stimulation of the respiratory burst with generation of free radicals,[13] liberation of products of the lipoxygenase pathway,[14] and increased Fc receptor activity.[15]

We have shown that FMLP contracts human airway smooth muscle in vitro.[16] Since this chemotactic peptide may play a part in the bronchoconstriction related to infection observed in patients with chronic bronchitis, we have carried out a preliminary investigation of the effects of nebulised FMLP on airway function and reactivity in human smokers and non-smokers.

Methods

Ten subjects recruited from the staff of the hospital

gave informed written consent to participation in this study. The protocol had been approved by the human ethics committees of the Royal North Shore and Concord Hospitals. Five subjects were non-smokers and five were current smokers; none gave a history of asthma. Baseline spirometry was performed with a Minato Autospirometer and the forced expiratory volume in one second (FEV_1) was expressed as a percentage of the predicted values published by Morris *et al.*[17]

All subjects underwent a histamine inhalation test based on the method of Chai *et al.*[18] A Rosenthal-French dosimeter (timing 0·6 seconds, pressure 138 kPa) and a DeVilbiss 646 nebuliser were used to deliver five breaths of saline and then doubling concentrations of histamine (0·03–8 mg/ml) while the subjects inhaled from functional residual capacity to total lung capacity. Immediately after each dose the FEV_1 was remeasured and repeated three minutes later. The procedure was stopped when the FEV_1 fell by 20% below the value obtained after inhalation of saline. The results were entered into a computer (PDP 11/73) and the $PC_{20}H$ (the concentration of histamine required to induce a 20% or greater fall in FEV_1) was determined by interpolation.

The FMLP (Sigma Chemical Company, St Louis, Missouri) was dissolved in 50% dimethylsulphoxide and 50% saline. Solutions containing doubling concentrations of 0·06–4 mg/ml FMLP were freshly prepared on each study day and administered with the dosimeter as described above. The DMSO-saline mixture was used as the control solution. Initially, subject 1 performed spirometry at three minute intervals for 15 minutes after each dose to establish the time course of the response. The FMLP induced bronchoconstriction reached a plateau at 3 minutes, so the FMLP dose-response curves were obtained in a manner identical to the performance of the histamine inhalation test (HIT). After the highest concentration of FMLP had been administered, the FEV_1 was remeasured every three minutes until it returned to

within 5% of the value obtained after inhalation of the control solution. The PC_{20} FMLP was determined in an identical manner to the $PC_{20}H$. In addition, four subjects serially inhaled five breaths of 50% dimethylsulphoxide and 50% saline every three minutes on five occasions.

All subjects had an FMLP dose-response curve performed on a different day from the histamine inhalation test. Subjects who had a PC_{20} FMLP of less than 4 mg/ml produced a second FMLP dose-response curve after the inhalation of nebulised ipratropium bromide (500 μg) on a separate day. Subject 8 declined a second FMLP exposure. Ipratropium bromide (500 μg) was also nebulised alone on a separate occasion with a repeat FEV_1 measurement 15 minutes later.

To determine whether FMLP inhalation is followed by airway hyperresponsiveness we gave a histamine inhalation test (up to 32 mg/ml histamine) to six additional subjects. This was followed by inhalations of 1·25 and 2·5 mg/ml FMLP. The histamine inhalation test was then repeated four and 24 hours later. Heart rate (radial pulse) and blood pressure were measured before and immediately after FMLP inhalation.

Results

The age, sex, FEV_1, and smoking histories of the subjects are shown in the table. There were six men and four women and all had normal or near normal lung function. The smokers were all current smokers and their cigarette consumption ranged from five to 22 pack years. None of the subjects was having bronchodilator or other drug treatment.

The $PC_{20}H$ was greater than 8 mg/ml in all of the first 10 subjects. The PC_{20} FMLP, determined in one subject only on three occasions one week apart, was 2·96, 2·05, and 3·14 mg/ml, indicating reproducibility within one doubling concentration. Eight of the 10 subjects responded to FMLP. Seven subjects returned

Baseline data and FMLP responses in 10 subjects

Subject No	Sex	Age (y)	FEV_1 (l)	FEV_1 (% pred)	Smoking (pack y)	PC_{20} FMLP (mg/ml)	PC_{20} FMLP after IB (mg/ml)
1	M	38	4·57	106	0	3·0	>4·0
2	M	40	3·98	97	0	1·0	3·3
3	F	29	2·41	84	0	0·7	>4·0
4	F	30	3·11	106	0	2·0	>4·0
5	M	40	2·78	77	0	4·0	NP
6	F	29	3·51	111	5	0·3	>4·0
7	F	30	2·54	82	9	>4·0	NP
8	M	31	4·08	100	10	0·4	NP
9	M	42	2·85	76	22	0·1	0·2
10	M	40	3·63	97	20	>4·0	NP

NP—not performed; IB—ipratropium bromide.
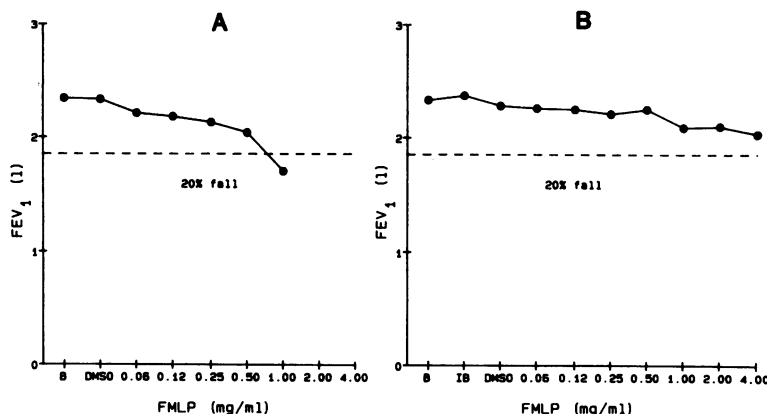
Formyl-methionyl-leucyl-phenylalanine (FMLP) dose-response curves in one subject (No 3). Panel A shows the FEV_1 response to FMLP with a PC_{20} FMLP of 0·7 mg/ml. Panel B shows the FEV_1 response to FMLP after nebulisation of 500 µg ipratropium bromide (IB) with a PC_{20} FMLP of > 4 mg/ml. B—baseline; DMSO—dimethylsulphoxide.

to within 5% of baseline FEV_1 in 18–24 minutes. Subject 2, however, had a prolonged response of 90 minutes' duration. Multiple inhalations of dimethylsulphoxide-saline did not result in bronchoconstriction (mean change in FEV_1 + 0·3%). Pretreatment with ipratropium bromide inhibited the response to FMLP (table). Typical response curves are shown in the figure. Ipratropium bromide itself produced minimal bronchodilatation with an average increase in FEV_1 of 2·4% 15 minutes after inhalation (mean (SEM) absolute increase in FEV_1 0·05 (0·04) l).

In addition to the bronchoconstrictor response the subjects developed other symptoms. Within 30 seconds of inhaling the lowest concentration of FMLP nine subjects developed considerable facial flushing, sweating, and in some cases conjunctival erythema and a feeling of light headedness. Cough also occurred frequently. These symptoms did not progress with increasing concentrations of FMLP but resolved over the next 6–9 minutes while increasing concentrations of FMLP were being administered. Subject 10, who also failed to develop bronchoconstriction in response to FMLP, did not show these features. Subjects 5 and 7, however, developed the flushing with little or no bronchoconstriction.

None of the six subjects participating in the study to see whether FMLP inhalation was followed by an increase in airway responsiveness had a 20% decline in FEV after 32 mg/ml histamine at baseline. The mean (SEM) fall in FEV_1 after FMLP was 11·4% (1·9%). A repeat histamine inhalation test at 4 and 24 hours failed to detect an increase in responsiveness, all subjects maintaining a $PC_{20}H$ of over 32 mg/ml.

Heart rate and blood pressure were not significantly different from baseline after FMLP inhalation at the time of the flushing response (mean (SEM) 79 (4·9) before and 88 (6·9) beats/min after; 127/83 before and 127/88 mmHg after).

Discussion

In previous work we have nebulised FMLP (up to 10 mg/ml) in rabbits and taken multiple serial sections of the bronchial tree. Although we observed increases in pulmonary resistance[19] in some of these animals, detailed histological examination failed to detect any evidence of airway inflammation. After this observation, we thought it justifiable to extend the studies to human subjects, although we limited the maximal concentration to 4 mg/ml.

There are several potential mechanisms for the bronchoconstriction in man. FMLP receptors could be present on airway smooth muscle. In the guinea pig the structure-function relations of synthetic oligopeptides for chemotactic and spasmogenic activity are identical.[20] Indeed, FMLP may bind to substance P receptors since the NH_2 terminal amino acid sequence of the undecapeptide substance P (phe-glu-leu-met-NH_2) resembles FMLP. In the rabbit substance P has been shown to bind to the FMLP receptor on the neutrophil.[21] Alternatively, FMLP may cause the release of substance P, known to be present in the vagus and fine nerves supplying the airways.[22] In vitro, however, capsaicin pretreatment failed to inhibit FMLP responses in human airways.[16] Clearly, studies with substance P antagonists in vitro would be useful to elucidate further any relationships with substance P. Since substance P may release acetylcholine at the neuromuscular junction[23] the bronchoconstriction induced by FMLP may have a cholinergic component.

In the present study the dose-response curves were shifted to the right after pretreatment with ipratropium bromide. Previous results in vitro, however, failed to show inhibition by atropine.[16] Alternatively, the efficacy of the anticholinergic drug in vivo may be related to inhibition of the effects of irritant receptors firing in response to FMLP. Certainly, cough frequently occurred in subjects after FMLP inhalation. The shift to the right of the FMLP-response curve after ipratropium bromide is unlikely to be simply a reflection of bronchodilatation since the subjects had normal baseline lung function and failed to respond to ipratropium bromide when this was tested separately.

Since substance P[24] and possibly FMLP[25] are vasodilators the flushing reaction observed in the present study may be explained by absorption from the airways of FMLP or systemic release of substance P, or both. We have measured heart rate and blood pressure after FMLP inhalation, however, and did not detect any change. The development of the flushing response at a lower concentration than that required for bronchoconstriction and its non-progressive nature with increasing concentrations of FMLP suggests a mechanism different from that of the bronchoconstriction.

If other mediators play a part, it is difficult to suggest likely possibilities. There is evidence that FMLP produces histamine release from human mast cells in vitro.[26] Airway smooth muscle contraction in vitro in response to FMLP was not, however, inhibited by H_1 antagonists in man[16] or guinea pig.[27] It has recently been shown that platelet activating factor is released from human polymorphs stimulated with FMLP.[28] Synthesis occurred within 2·5 minutes, however, and release occurred 5 minutes after stimulation. These delayed responses therefore fail to explain the in vivo or in vitro data.

The lack of development of airway hyperresponsiveness after inhalation of FMLP is different from the effect of inhaled chemotactic factors in experimental animals. Irvin *et al*[29] recently showed that inhalation of activated complement fragments was followed by hyperresponsiveness to histamine. Lack of hyperresponsiveness may indicate a direct bronchoconstrictor or irritant effect, or both, without the intervention of any effector cells or other mediators.

There are important clinical implications of the findings in this study. During acute infective exacerbation in patients with chronic bronchitis airways resistance increases. This may be due to inflammatory changes in the airways, oedema and mucus causing airway obstruction. Clinically, however, increases in airways resistance are commonly found when sputum becomes coloured and infected even when sputum volumes are not increased. Several potential mechanisms for this observation have been considered in the past, including production of endotoxin by *Haemophilus influenzae*[30] causing bronchoconstriction, enhanced α-adrenergic responsiveness induced by endotoxin in patients with chronic bronchitis,[31] and mediator release induced by bacteria.[32] *Streptococcus pneumoniae* is capable of producing chemotactic factors.[3] This has not, however, been established for *H influenzae*. Nevertheless, the demonstration of the potent bronchoconstrictor response to FMLP in the human subjects in vivo raises the possibility that such a mechanism may be operating during infective exacerbations in patients with chronic bronchitis.

References

1 Harris H. Mobilization of defensive cells in inflammatory tissue. *Bact Rev* 1960;**24**:3–15.
2 Keller HV, Sorkin E. Studies on chemotaxis: V. On the chemotactic effect of bacteria. *Int Arch Allergy* 1967;**31**:505–17.
3 Ward PA, Lepow IH, Newman LJ. Bacterial factors chemotactic for polymorphonuclear leukocytes. *Am J Pathol* 1968;**52**:725–36.
4 Walker WS, Bartlet RC, Kurtz HM. Isolation and partial characterization of a staphylococcal leukocyte cytotoxin. *J Bacteriol* 1969;**97**:1005–8.
5 Schiffman E, Showell HV, Corcoran BA, Ward PA, Smith E, Becker EL. The isolation and partial characterization of neutrophil chemotactic factors from *Escherichia coli. J Immunol* 1975;**114**:1831–7.
6 Showell HJ, Freer RJ, Zigmond SH, Schiffmann E, Kaswanikumar S, Corcoran B, Becker EL. The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. *J Exp Med* 1976;**143**:1154–69.
7 Niedel J. Detergent solubilization of the formyl peptide chemotactic receptor. Strategy based on covalent affinity labelling. *J Biol Chem* 1980;**256**:9295–9.
8 Schmitt M, Painter RG, Jesaitis AJ, Preissner K, Sklar LA, Cochrane CG. Photoaffinity labelling of the N-formyl peptide receptor binding site of intact human polymorphonuclear leukocytes. A label suitable for following the fate of the receptor-ligand complex. *J Biol Chem* 1983;**258**:649–54.
9 Goetzl E, Forster DW, Goldman DW. Isolation and partial characterization of membrane protein constituents of human neutrophil receptors for chemotactic formylmethionyl peptides. *Biochemistry* 1981;**20**:5717–22.
10 Freer RJ, Day AR, Muthukumaraswamy N, Pinon D, Wu A, Showell HJ, Becker EL. Formyl peptide chemoattractants: A model of the receptor on rabbit neutrophils. *Biochemistry* 1982; **21**:257–63.
11 Smith RJ, Iden SS. Pharmacological modulation of chemotactic factor elicited release of granule-associated enzymes from human neutrophils. Effects of

prostaglandins, non-steroid anti-inflammatory agents and corticosteroids. *Biochem Pharmacol* 1980;**79**:2389–95.

12 Smith RJ, Wierenga W, Iden SS. Characteristics of *N*-formyl-methionyl-leucyl-phenylalanine as an inducer of lysosomal enzyme release from human neutrophils. *Inflammation* 1980;**4**:73–88.

13 Wong K, Freund K. Inhibition of the *N*-formyl-methionyl-leucyl-phenylalanine induced respiratory burst in human neutrophils by adrenergic agonists and prostaglandins of the E series. *Can J Physiol Pharmacol* 1981;**59**:915–20.

14 Jubiz W, Radmark O, Malmsten C, *et al.* A novel leukotriene produced by stimulation of leukocytes with formylmethionyl-leucyl-phenylalanine. *J Biol Chem* 1982;**257**:6106–10.

15 Rossman MD, Cassizzi AM, Schreiber AD, Daniele RP. Pulmonary defense mechanisms. Modulation of Fc receptor activity in alveolar macrophages and other phagocytic cells by N-formylpeptides. *Am Rev Respir Dis* 1982;**126**:136–41.

16 Armour CL, Black JL, Johnson PRA, Vincenc KS, Berend N. Formyl-peptide induced contraction of human airways in vitro. *J Appl Physiol* 1986;**60**:141–6.

17 Morris JF, Koski A, Johnson LC. Spirometric standards for healthy non-smoking adults. *Am Rev Respir Dis* 1971;**103**:57–67.

18 Chai H, Farr RS, Froehlich LA, *et al.* Standardization of bronchial inhalation challenger procedures. *J Allergy Clin Immunol* 1975;**56**:323–7.

19 Berend N, Armour CL, Black JL. Formyl-methionyl-leucyl-phenylalanine causes bronchoconstriction in rabbits. *Agents Actions* 1985;**17**:466–71.

20 Marasco WA, Fantone JC, Ward PA. Spasmogenic activity of chemotactic *N*-formylated oligopeptides: identity of structure-function relationships for chemotactic and spasmogenic activities. *Proc Natl Acad Sci* 1982;**79**:7470–3.

21 Marasco WA, Showell JH, Becker EL. Substance P binds to the formylpeptide chemotaxis receptor on the rabbit neutrophil. *Biochem Biophys Res Comm* 1981;**99**:1065–72.

22 Wharton J, Polak JM, Bloom SR, Will JA, Brown MR, Pearse AGE. Substance P-like immunoreactive nerves in mammalian lung. *Invest Cell Pathol* 1979;**2**:3–10.

23 Tanaka DT, Grunstein MM. Mechanism of substance P-induced contraction of rabbit airway smooth muscle. *J Appl Physiol* 1984;**57**:1551–7.

24 Hallberg D, Pernow B. Effect of substance P on various vascular beds in the dog. *Acta Physiol Scand* 1975;**93**:277–85.

25 Saito H, Matsuo M, Naminohira S, Sakai T, Kitagawa H, Hirata AA. Effect of *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine and its analogue on blood pressure. *J Pharmacobiodyn* 1983;**6**:141–4.

26 Hook WA, Schiffmann E, Aswanikumar S, Siraganian RP. Histamine release by chemotactic, formyl methionyl-containing peptides. *J Immunol* 1976;**117**:594–6.

27 Hamel R, Ford-Hutchinson AW, Lord A, Cirino M. Bronchoconstriction induced by *N*-formyl-methionyl-leucyl-phenylalanine in the guinea pig involvement of arachidonic acid metabolites. *Prostaglandins* 1984;**28**:43–56.

28 Ludwig JC, McManus LM, Clark PO, Hanahan DJ, Pickard RN. Modulation of platelet-activating factor (PAF) synthesis and release from human polymorphonuclear leukocytes (PMN): role of extracellular calcium. *Arch Biochem Biophys* 1984;**232**:102–10.

29 Irvin CG, Berend N, Henson PM. Airways hyperreactivity and inflammation produced by aerosolization of human C5a des Arg. *Am Rev Respir Dis* 1986;**134**:777–83.

30 Van der Zwan JC, Orie NGM, Kauffman HF, Wiers PWJ, De Vries K. Bronchial obstructive reactions after inhalation with endotoxin and precipitinogens of *Haemophilus influenzae* in patients with chronic non-specific lung disease. *Clin Allergy* 1982;**12**:547–59.

31 Simonsson BG, Svedmyr N, Skoogh BE, Andersson R, Bergh NP. In vivo and in vitro studies on alpha-receptors in human airways. Potentiation with bacterial endotoxin. *Scand J Respir Dis* 1972;**53**:227–36.

32 Martin RR, White A. The in vitro release of leukocyte histamine by staphylococcal antigens. *J Immunol* 1969;**102**:437–41.