Vitamin E attenuates the injurious effects of bioactive phospholipids on human ciliated epithelium *in vitro*

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ABSTRACT: Bioactive phospholipids (PL), particularly lysophosphatidylcholine (LPC), are being increasingly implicated in the pathogenesis of various acute and chronic inflammatory disorders, particularly those of the airways, while there is emerging evidence that vitamin E may function as a natural antagonist of these lipid mediators of inflammation. The aims of this study were to document the effects of vitamin E on the inhibition of ciliary beating and damage to structural integrity of human ciliated epithelium induced by the PL, platelet-activating factor (PAF), lyso-PAF and LPC *in vitro* in relation to the anti-oxidative and membrane-stabilizing properties of the vitamin.

Ciliary beat frequency was measured by a phototransistor technique, and damage to structural integrity assessed by a visual-scoring index, while superoxide production by polymorphonuclear leukocytes and membrane-stabilizing potential were measured using lucigenin-enhanced chemiluminescence and haemolytic procedures, respectively.

All three PL caused inhibition of ciliary beating and structural damage to human ciliated epithelium by membrane-directed cytotoxic mechanisms, which were potentiated by human polymorphonuclear leukocytes due to induction of oxidant-mediated injury. Both direct and phagocyte-inflicted epithelial injury was attenuated by vitamin E. In haemolytic and chemiluminescence assays, vitamin E neutralized both the membrane-destabilizing and pro-oxidative actions of all three PL, while spectro-photometric analysis of mixtures of vitamin E with PAF, lyso-PAF and LPC revealed alterations in peak intensity, as well as peak shifts, indicative of physicochemical interactions between the vitamin and the PL.

Vitamin E status may be a determinant of susceptibility to phospholipid-mediated airway inflammation and damage.
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Activation of phospholipase A_2 in immune and inflammatory cells results in the production of bioactive phospholipids (PL), such as lysophosphatidylcholine (LPC), and may also initiate the production of platelet-activating factor (PAF) and lyso-PAF [1]. Both LPC and PAF possess pro-inflammatory properties [1–5] compatible with a role for these bioactive PL in the immunopathogenesis of a wide variety of acute [6–9] and chronic inflammatory disorders of both infective and noninfective origin. These molecules appear to be particularly important as mediators of inflammatory conditions of the airways, including bronchial asthma [10–12].

In spite of the well-recognized pro-inflammatory actions of PAF, most of which are thought to be receptor-mediated [1, 13], therapeutic interventions based on pharmacological antagonism of PAF receptors on immune and inflammatory cells have been disappointing in disorders such as acute respiratory distress syndrome [14] and bronchial asthma [10, 15, 16]. These observations suggest either that the *in vivo*

pro-inflammatory potential of PAF is overrated [16], or that the cytotoxic and pro-inflammatory actions of PAF may be independent of PAF receptors, resulting from nonspecific membrane disruptive mechanisms as appears to be the case with LPC [17, 18].

Interestingly, the membrane-destabilizing actions of LPC *in vitro* are known to be neutralized by vitamin E (alpha tocopherol), apparently as a consequence of physicochemical interactions between these agents [19, 20], while deficiency of this vitamin has been reported to result in increased synthesis of PAF by activated neutrophils [21]. These cytoprotective and anti-inflammatory properties of vitamin E, particularly in relation to neutralization of bioactive PL, may be of particular importance in the airways and may explain the findings of several epidemiological studies of an inverse relationship between vitamin E intake and pulmonary function [22–25], as well as the risk for developing adult-onset asthma [26] and wheeze [27]. However, in most of these studies it has been suggested that the beneficial effects of vitamin E may be

mediated through anti-oxidative activity as opposed to the other aforementioned anti-inflammatory actions of this vitamin.

The current study was undertaken to investigate and compare the effects of LPC and PAF on the structure and function of ciliated respiratory epithelium, a first-line host defense against airborne microbial and viral pathogens, pollutants and allergens, and to elucidate the possible function of vitamin E as a natural antagonist of both LPC and PAF.

Materials and methods

Chemicals and reagents

Vitamin E (DL-α-tocopherol, F. Hoffmann-La Roche, Basel, Switzerland) was solubilized in dimethyl sulphoxide and added directly to the cell cultures as described below with the appropriate solvent controls. Unless indicated, all other chemicals and reagents were purchased from the Sigma Chemical Co., St Louis, MO, USA.

Polymorphonuclear leukocytes

Human polymorphonuclear leukocytes (PMNL) were obtained from heparinized (5 units of preservative-free heparin·mL^{-1}) venous blood of healthy adult volunteers and separated from mononuclear leukocytes by centrifugation on Histopaque® 1,077 cushions at 400×g for 25 min at room temperature. The resultant pellet was suspended in phosphate-buffered saline (PBS; 0.15 M, pH 7.4) and sedimented with 3% gelatin for 15 min at 37°C to remove most of the erythrocytes. After centrifugation, erythrocytes were removed by selective lysis with 0.84% ammonium chloride at 4°C for 10 min. The PMNL, which were routinely of high purity and viability, were resuspended to 1×10^7 cells·mL^{-1} in PBS and held on ice until used. The final concentration of PMNL used in the experiments on human ciliated epithelium described below, was 1×10^6 cells·mL^{-1}. Preliminary experiments showed that at this concentration, PMNL alone had no effect on ciliated epithelium over the time course of the experiments.

Human ciliated epithelium

Human ciliated epithelium was obtained by brushing the inferior nasal turbinate of healthy human volunteers, using a 2 mm bronchoscopy cytology brush, as described previously [28]. Permission to conduct the study was obtained from the Committee for Research on Human Subjects of the University of the Witwatersrand, with the requirement for verbal informed consent from the volunteers. On each occasion, strips of epithelium were obtained from a single volunteer and these were suspended in Hanks balanced salt solution (HBSS; pH 7.4, Highveld Biological, South Africa). For each experiment, the epithelial strips in HBSS were divided into three or four 2 mL aliquots using a Pasteur pipette. Each aliquot was centrifuged at 150×g for 10 min. Thereafter, the supernatant from each aliquot was aspirated and replaced by 800 μL of either control or test solution.

A closed microscope coverslip-slide preparation was prepared for each specimen as described previously, and the specimens were coded by an independent observer. The preparations were then incubated for 10 min at 37°C, then placed on an electronically controlled warm stage (Sensicon, LHE Laboratories, UK) set to 37°C, on a Leitz Orthoplan phase contrast microscope (Leitz, Wetzlar, Germany). In each specimen, 6–10 strips of epithelium were selected and one or two areas on each of these strips were marked for later identification. Ciliary beat frequency (CBF) was measured at these sites by a photo-transistor technique, described previously [29]. In brief, the strips of epithelium were orientated such that their beating interrupted a beam of light across their pathway, the modulation in light intensity being sensed by a photo-transistor. The signal generated was amplified and optimized on an oscilloscope screen and was then converted into a digital reading of CBF. Ten readings were taken from each of the specimens initially and every hour for 4 h. The mean CBF at each time point was calculated from these 10 readings. The values are expressed as mean±SE. The per cent ciliary slowing (%CS) was calculated as the mean control CBF minus the mean test CBF divided by the mean control CBF×100.

Damage to the structural integrity of the epithelial strips (epithelial damage (ED)) was assessed by a visual scoring index, similar to that described previously [30]. In short, the presence of disruption of the normal integrity of the epithelial surface (presence of irregular contour and break-up of the normally smooth outline) was recorded by the same investigator as being either absent or present at the same sites at which the recordings of CBF were undertaken.

These procedures were used to investigate the following. 1) The direct effects of PL alone on human ciliated epithelium in the absence and presence of vitamin E. In these experiments, controls contained epithelial strips in HBSS alone, while test preparations contained individual epithelial strips exposed to the individual PL, namely PAF, lyso-PAF and LPC (5 μg·mL^{-1}) in the absence or presence of vitamin E (20 μg·mL^{-1}). Epithelial strips were preincubated with vitamin E at 37°C for 30 min prior to the addition of PL. Preliminary experiments had demonstrated that at concentrations up to 50 μg·mL^{-1}, vitamin E *per se* had no effect on CBF or structural integrity of human ciliated epithelium over 4 h. 2) The effects of PL in combination with PMNL on human ciliated epithelium in the absence and presence of vitamin E. In these experiments, control preparations contained epithelial strips and PMNL (1×10^6 cells·mL^{-1}, final) in HBSS alone and test preparations contained epithelial strips exposed to PL (1.25 μg·mL^{-1})-treated PMNL (1×10^6 cells·mL^{-1}). To study the effects of vitamin E, two additional systems were used. In the first of these, the epithelial strips were preincubated with vitamin E (37°C for 30 min; final

concentration 10 $\mu g \cdot mL^{-1}$) prior to the addition of the PL (1.25 $\mu g \cdot mL^{-1}$)-treated PMNL (1×10^6 cells$\cdot mL^{-1}$), while in the second system the PMNL (1×10^6 cells$\cdot mL^{-1}$) were preincubated with vitamin E (37°C for 30 min; final concentration of 10 $\mu g \cdot mL^{-1}$), prior to the addition of PL (1.25 $\mu g \cdot mL^{-1}$) and immediate mixing with the epithelial strips. Preliminary experiments had demonstrated that concentrations of PL of 5 $\mu g \cdot mL^{-1}$ (in experiments without PMNL) and 1.25 $\mu g \cdot mL^{-1}$ (in experiments with PMNL) were optimum for studying their effects on ciliated epithelium (*i.e.* there were sufficient effects on ciliated epithelium to be able to monitor the injurious effects of the PL with and without vitamin E, without complete disruption of the tissue).

Superoxide generation by polymorphonuclear leukocytes

This was measured using a lucigenin (bis-*N*-methylacridinium nitrate)-enhanced chemiluminescence (LECL) method. PMNL were pre-incubated for 15 min at room temperature in 900 μL HBSS containing 0.2 mM lucigenin in the presence and absence of vitamin E (10 and 20 $\mu g \cdot mL^{-1}$) followed by 15 min at 37°C. Spontaneous and stimulus-activated LECL responses were then recorded in an LKB Wallac 1251 chemiluminometer (Turku, Finland) after the addition of 100 μl of PAF, lyso-PAF or LPC (10 $\mu g \cdot mL^{-1}$). LECL readings were integrated for 5-s intervals and recorded as millivolts$\times$seconds^{-1} ($mV \cdot s^{-1}$).

Membrane stabilization

The membrane-stabilizing potential of vitamin E was measured using a haemolytic assay. Sheep erythrocytes were triple-washed and resuspended to 5% in HBSS. The erythrocytes (final concentration of 0.5%) were then coincubated with vitamin E (2.5–20 $\mu g \cdot mL^{-1}$) for 30 min at 37°C, followed by the addition of the membrane-destabilizing bioactive phospholipids, LPC, PAF and lyso-PAF at concentrations of 5–7 $\mu g \cdot mL^{-1}$, which caused partial haemolysis. After 5 min, intact erythrocytes were removed by centrifugation and the supernatant assayed

spectrophotometrically at 405 nm for haemoglobin content.

Physicochemical interactions of vitamin E with platelet-activating factor, lyso-platelet-activating factor and lysophosphatidylcholine

A Hitachi 150–20 double beam ultraviolet (UV) spectrophotometer was used to measure possible complex-forming interactions between vitamin E and PAF or lyso-PAF as have previously been reported to occur between LPC and vitamin E [19, 31]. The UV absorption spectra of the PL alone (1 $mg \cdot mL^{-1}$), blanked against the ethanol solvent in the reference cuvette, were compared (with respect to peak maxima and peak shifts) with those of mixtures of identical concentrations of each PL with vitamin E (0.1 $mg \cdot mL^{-1}$) using a reference cuvette containing vitamin E alone in the ethanol solvent. In an additional series of experiments, differences in the UV spectra of vitamin E (0.01 $mg \cdot mL^{-1}$) following mixing with the PL (0.1 $mg \cdot mL^{-1}$) were measured using a reference system containing the PL only.

Statistical analysis

The results of each series of experiments are expressed as the mean$\pm$SE. Where appropriate, levels of statistical significance were calculated by paired t-test. The effects on CBF and amount of ED were compared in control and test preparations by means of the 2-tailed U-test of Mann-Whitney. Each series of experiments was conducted six times. A p-value of <0.05 was considered to be significant.

Results

The direct effects of the bioactive phospholipids on human ciliated epithelium in the absence and presence of vitamin E

As shown in table 1 and figures 1 and 2, all three bioactive phospholipids, PAF, lyso-PAF and LPC (final concentration 5 $\mu g \cdot mL^{-1}$) caused significant

Table 1. – The mean$\pm$SE ciliary beat frequency (CBF) and per cent ciliary slowing (%CS) and per cent epithelial damage (%ED) in human ciliated respiratory epithelium exposed to bioactive phospholipids (PL) in the absence and presence of vitamin E

	Control strips* in HBSS alone		Epithelial strips exposed to PL alone 5 $\mu g \cdot mL^{-1}$			Epithelial strips preincubated with vitamin E (20 $\mu g \cdot mL^{-1}$) prior to exposure to PL		
	CBF Hz	%ED	CBF Hz	%CS	%ED	CBF Hz	%CS	%ED
PAF[#]	11.4±0.23	0	8.3±0.49[+]	27	43[+]	11.0±0.27[§]	4	7[§]
Lyso-PAF	11.7±0.22	0	7.9±0.68[+]	32	38[+]	11.0±0.36[§]	6	5[§]
LPC[¶]	11.5±0.11	0	9.3±0.28[+]	19	37[+]	11.1±0.31[§]	3	2[§]

*: control strips contained no PL; [#]: platelet-activating factor (PAF); [¶]: lysophosphatidylcholine (LPC). HBSS: Hanks balanced salt solution; [+]: p<0.005 *versus* control; [§]: p<0.01–p<0.005 compared to the corresponding systems without vitamin E, but not significantly different from the control systems. There were no significant differences between the effects of the individual PL on CBF and ED.
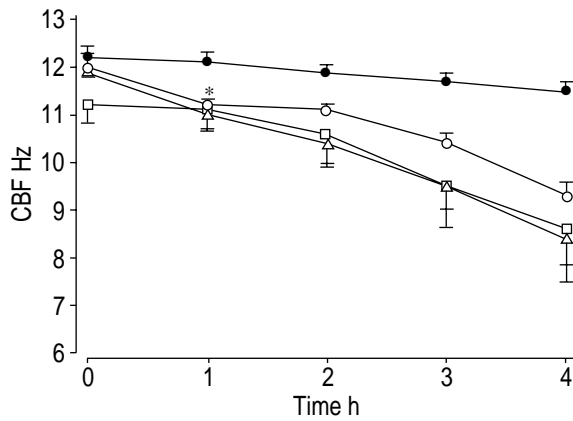
Fig. 1.–Effects of lysophosphatidylcholine (○), platelet-activating factor (PAF) (△) and lyso-PAF (□) (all at 5 µg·mL⁻¹) on the ciliary beat frequency (CBF) of human ciliated epithelium over 4 h *in vitro*. Data from six experiments with each phospholipid are presented as the mean±SE. The results of the control experiments have been combined for clarity. ●: control; *: denotes time from which CBF of test systems is significantly different (p<0.05) from the control system.

slowing of CBF and ED in human ciliated epithelium after 4 h. Figure 2 is a photomicrograph of the epithelial strip incubated in tissue culture fluid alone (fig. 2a) compared with a paired epithelial strip incubated for 4 h with PAF (final concentration 5 µg·mL⁻¹; fig. 2b). The latter demonstrates the damage to the structural integrity of epithelial strips following exposure to PL, which was assessed using the visual scoring
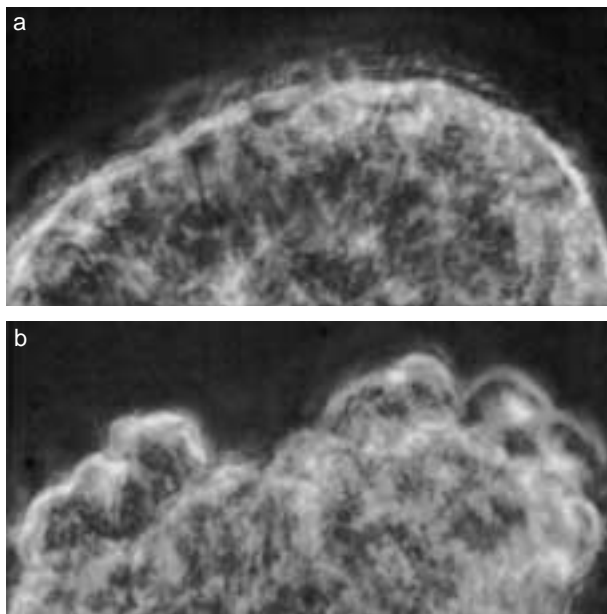

Fig. 2.–A photomicrograph (phase contrast, original magnification ×400) of the appearance of a control epithelial strip a) incubated in tissue culture fluid alone and b) a test epithelial strip after 4-h exposure to platelet-activating factor (5 µg·mL⁻¹ final concentration). The control shows a well ciliated surface with smooth outline. The test shows the presence of irregular contour and break-up of the normally smooth outline with poor ability to visualize the cilia in many areas.

index. Preincubation of epithelial strips with vitamin E (final concentration 20 µg·mL⁻¹) was associated with significant antagonism of LPC-, PAF- and lyso-PAF-mediated slowing of CBF and ED, as shown in table 1.

The effects of bioactive phospholipids in combination with polymorphonuclear leukocytes on human ciliated epithelium in the absence and presence of vitamin E

These results are shown in table 2. All three bioactive phospholipids, PAF, lyso-PAF and LPC (final concentration of 1.25 µg·mL⁻¹) in the presence of PMNL (1×10⁶ cells·mL⁻¹) caused significant ciliary slowing and epithelial damage after 4 h. These effects were significantly antagonized by preincubation of the epithelial strips, as well as the PMNL, with vitamin E (10 µg·mL⁻¹).

Superoxide generation

The effects of LPC, PAF and lyso-PAF (10 µg·mL⁻¹) on superoxide production by neutrophils are shown in figure 3. All three bioactive phospholipids, but LPC and PAF in particular, activated the production of superoxide by neutrophils, which was antagonized (p<0.05) by preincubation of the cells with vitamin E (10 and 20 µg·mL⁻¹).

Membrane stabilization

The effects of vitamin E on the lysis of sheep erythrocytes mediated by the bioactive phospholipids LPC, PAF and lyso-PAF are shown in figure 4. At the concentrations tested (2.5–20 µg·mL⁻¹) vitamin E caused statistically significant (p<0.05) protection of the erythrocytes against haemolysis induced by all three membrane-destabilizing phospholipids.

Interactions of vitamin E with the bioactive phospholipids

The results shown in table 3 are those for the spectrophotometric analysis of mixtures of vitamin E (0.1 mg·mL⁻¹) with PAF, lyso-PAF and LPC (1 mg·mL⁻¹) with vitamin E alone in the reference cuvette. These demonstrate major reductions in the intensity of the major PL peaks at 215–218 nm, as well as peak shifts, compatible with interactions of vitamin E with PAF, lyso-PAF and LPC. The results of the converse experiments in which the UV spectrum of vitamin E alone (0.01 mg·mL⁻¹) was compared with that of mixtures of vitamin E with PAF (0.1 mg·mL⁻¹) using a reference cuvette with PAF alone are shown in figure 5. Addition of PAF to vitamin E resulted in a major reduction in the intensity of the vitamin E peak at 201 nm.

Table 2. – Mean±SE ciliary beat frequency (CBF) and per cent ciliary slowing (%CS) and per cent epithelial damage (%ED) in human ciliated respiratory epithelium exposed to bioactive phospholipid (PL)-sensitized polymorphonuclear leukocytes (PMNL; 1×10^6 cells·mL^{-1}) in the presence and absence of vitamin E (final concentration 10 µg·mL^{-1})

	PAF			Lyso-PAF			LPC		
	CBF Hz	%CS	%ED	CBF Hz	%CS	%ED	CBF Hz	%CS	%ED
Control epithelial strips	11.2+0.10		0	11.6+0.03		0	11.9+0.15		0
Epithelial strips exposed to PL (1.25 µg·mL^{-1})-sensitized PMNL[#]	9.0+0.32	20	33	9.2+0.85	21	32	9.4+0.16	21	31
Preincubation of epithelial strips with vitamin E[+]	11.5+0.25	0	0	11.1+0.05	4	0	11.4+0.10	4	0
Preincubation of PMNL with vitamin E[+]	11.5+0.00	0	0	11.4+0.10	2	0	11.7+0.15	2	0

PAF: platelet-activating factor; LPC: lysophosphatidylcholine; [#]: p<0.005 *versus* control; [+]: p<0.005 compared to the corresponding systems without vitamin E, but not significantly different from the control systems. There were no significant differences between systems containing the different PL.

Discussion

In the clinical setting it appears that vitamin E may modulate lung function, particularly in the elderly, and protect against deterioration of lung function in both smokers and nonsmokers [22–25]. In terms of asthma, dietary deficiency of vitamin E has been shown to correlate with adult-onset wheeze [27], while dietary supplementation may protect against the development of asthma in adults [26]. More recently it has been documented that airway lining fluid in asthmatics is deficient in vitamin E (and vitamin C), which together with increased levels of oxidized glutathione suggests that the airways of asthmatics are subjected to increased oxidative stress [32]. The possible mechanisms of interaction of vitamin E with the PL, important putative mediators in asthma and other inflammatory disorders of the airways, were investigated in human ciliated epithelium *in vitro*.

In the current study, PAF, lyso-PAF and LPC caused slowing of ciliary beating of human ciliated

respiratory epithelium, as well as damage to epithelial integrity which was progressive over 4 h. At the concentration used (5 µg·mL^{-1}), these three PL appeared to be equipotent with respect to inhibition of CBF and epithelial damage. PAF, lyso-PAF and LPC, at the same concentrations which caused injury to and dysfunction of ciliated epithelial cells, also
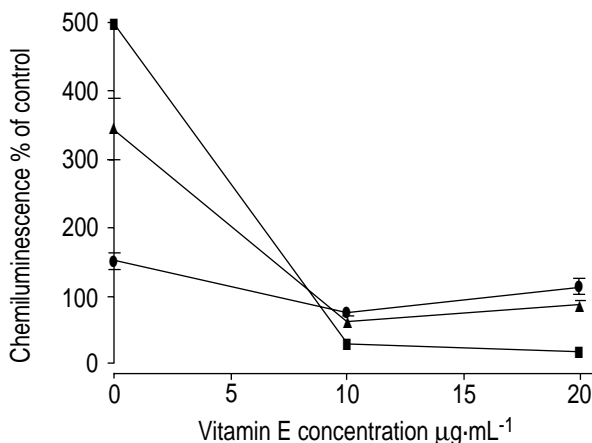

Fig. 4.–Effects of vitamin E (2.5–20 µg·mL^{-1}) on lysophosphatidylcholine (LPC)- (■), platelet-activating factor (PAF)- (▲) and lyso-PAF (●) (5–7 µg·mL^{-1})-mediated lysis of sheep erythrocytes. The results of 12 experiments are presented as the mean percentage haemolysis±SE.

Table 3. – Spectophotometric analysis of mixtures of lysophosphatidylcholine (LPC), platelet-activating factor (PAF) and lyso-PAF (1 mg·mL^{-1}) individually and in combination with vitamin E (VE) (0.1 mg·mL^{-1})

	Absorption maxima nm		OD values	
	Without VE	With VE	Without VE	With VE
LPC	202	219	0.67	0.32
PAF	202	226	1.33	0.25
Lyso-PAF	202	216	0.44	0.15

OD: optical density.

Fig. 3.–Effects of vitamin E (10 and 20 µg·mL^{-1}) on superoxide production by lysophosphatidylcholine (LPC)- (■), platelet-activating factor (PAF)- (▲) and lyso-PAF (●) (all at 10 µg·mL^{-1})-activated polymorphonuclear leukocytes. The results of three experiments each with 3–5 determinations are presented as the mean±SE of the unstimulated control systems for which the absolute value was 554.2±56.6 millivolts×seconds^{-1}.
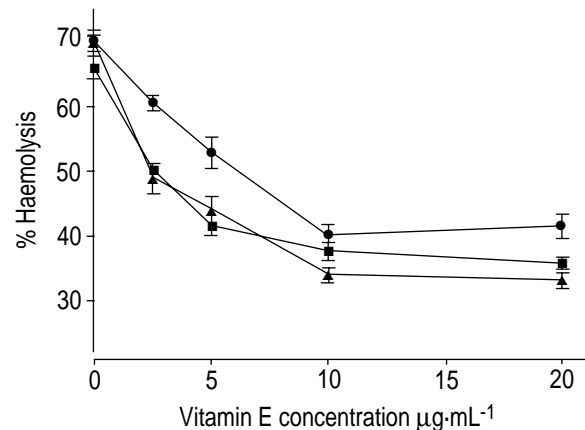
Fig. 5. – Ultraviolet spectra of vitamin E (——) alone at 0.01 mg·mL^{-1} and in combination with 0.1 mg·mL^{-1} PAF (----).

lysed sheep erythrocytes. These observations are compatible with a common mechanism of injury to both epithelial cells and erythrocytes, probably by non-specific membrane damage due to the detergent properties of the PL. The authors have previously reported that PL-mediated damage to ciliated respiratory epithelial cells (in the absence of added phagocytes) is not mediated by reactive oxidants generated by contaminating phagocytes in nasal epithelium, since no protection was provided by catalase in combination with superoxide dismutase [33]. Unlike the situation with erythrocytes, however, in which damage (cytolysis) was observed rapidly following exposure to the PL, epithelial damage and ciliary dysfunction occurred more slowly, being progressive over the 4-h incubation period. These observations suggest that ciliated epithelial cells may be more resistant to the cytolytic effects of PL than erythrocytes, with cellular dysfunction and damage resulting primarily from PL-mediated interference with plasma membrane cation transporters such as Ca^{2+}-adenosine triphosphatase (ATPase) and Na^+, K^+-ATPase [18, 20].

These direct, injurious effects of the PL on ciliated epithelium and erythrocytes were antagonized by preincubation of the strips or red cells with vitamin E. It has previously been reported that vitamin E complexes with, and neutralizes, LPC through two types of interaction, namely formation of a hydrogen bond between the vitamin E chromanol nucleus hydroxyl group and the carboxyl group of the lyso-phospholipid, and interaction of the acyl chains of the lysophospholipid with the chromanol nucleus methyl groups of vitamin E [17]. In the current study, the physicochemical interactions between vitamin E and LPC have been confirmed. An important and novel observation, however, was the demonstration of the ability of this vitamin to complex with both PAF and lyso-PAF. Although the exact nature of the physico-chemical interactions which occur between vitamin E and PAF/lyso-PAF remain to be established, the observations suggest that vitamin E may be a natural antagonist of LPC, PAF and lyso-PAF.

Several lines of evidence suggest that the observed direct, cytotoxic/cytolytic effects of PAF on ciliated respiratory epithelium and erythrocytes are not mediated via PAF receptors. These are: 1) these cell types do not express PAF receptors [1]; 2) in these studies lyso-PAF, which does not interact with PAF receptors [1], appeared to be equivalent to PAF with respect to cytotoxic/cytolytic potential; 3) the concentrations of PAF at which these effects were observed are considerably higher than those which activate PAF receptors [1]; and 4) hexanolamine-PAF, a PAF-receptor antagonist [34], did not antagonize, but rather potentiated, both the haemolytic and CBF-inhibitory actions of PAF (data not included).

The injurious effects of the PL on CBF and structural integrity of respiratory epithelium were potentiated by adding PMNL to the epithelial strips with significant injury detected at 1.25 µg·mL^{-1} of the PL, with no clear differences between LPC, PAF and lyso-PAF. It has previously been reported that in this setting, injury to the ciliated epithelium is indirect, being mediated predominantly by reactive oxidants originating from the PL-treated PMNL [33]. The three bioactive PL were equally effective, which was somewhat surprising since lyso-PAF was less potent than PAF and LPC with respect to activation of super-oxide production by neutrophils. This suggests that the priming effects of the PL on neutrophil pro-inflammatory activities may be more relevant in this experimental setting [35]. Interestingly, pretreatment of either the epithelial strips or the PMNL with vitamin E almost completely prevented the decrease in CBF and epithelial injury which accompanied addition of the PL to the system. Since vitamin E is an ineffective scavenger of phagocyte-derived reactive oxidants [36], the anti-oxidative interactions of this agent with neutrophils are probably due to inhibition of transductional events involved in activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. These include inhibition of protein kinase C [37], as well as interference with the phosphorylation and translocation from the cytosol to the plasma membrane of p47 Phox, an integral component of the activated oxidase [38]. In addition, the protein motions, both lateral and rotational, which are required for both the assembly and activity of NADPH oxidase in the phagocyte membrane [39], may be restricted by the membrane-stabilizing actions of the vitamin [40].

As shown previously, oxidant-mediated damage to respiratory epithelium is not instantaneous, but occurs progressively over several hours [41] as a result of depletion of intracellular adenosine triphosphate (ATP) by two mechanisms; firstly by oxidant-mediated inactivation of glycolytic enzymes, especially glyceraldehyde-3-phosphate dehydrogenase [42], and secondly by consumption of nicotinamide adenine dinucleotide (NAD) consequent to oxidant-mediated damage to deoxyribonucleic acid (DNA) and activation of the DNA repair enzyme, poly-ADP-ribose polymerase [43].

Although the concentrations of PAF and lyso-PAF used in the present study are considerably higher than those found in the airways of allergen-challenged asthmatics, those of LPC are certainly within the

range (12.5–100 µg·mL^{-1}) which have been reported to occur in this situation [12], as well as at other sites of inflammation, such as the ischemic myocardium [44]. This suggests that LPC is probably the most biologically relevant, at least with respect to cytotoxicity, of the three PL used in the current study. Aside from cytotoxic/cytolytic properties, the pro-inflammatory actions of LPC include induction of mast cell secretion [2], upregulation of the endothelial adhesion molecules, P-selectin and ICAM-1 [3], activation of protein kinase C [4] and PIP$_3$ kinase [5], as well as activation of superoxide production by neutrophils [5], as described in the present study. The vitamin E status of an individual may therefore be an important determinant of the reactivity of LPC. Although vitamin E is present in airway epithelial lining fluid and can be increased by oral supplementation, the exact concentrations of the vitamin in this extracellular compartment are difficult to determine, but may be lower than those in serum [32, 45]. However, in epithelial lining fluid the PL-neutralizing potential of vitamin E may be enhanced by the presence of surfactant which also possesses PL-buffering properties [46].

While most of the PAF generated by activated immune and inflammatory cells is retained intracellularly [1], that which is released extracellularly can amplify inflammatory responses by interacting with PAF receptors on target cells, particularly eosinophils [1] and neutrophils [47, 48]. The disappointing responses to PAF-receptor antagonists in patients with bronchial asthma [10, 15, 16] and acute respiratory distress syndrome [14] have suggested that PAF may be a minor contributor to the harmful inflammatory responses in these conditions. However, recent findings have suggested that this may not be the case [48] and that the poor anti-inflammatory efficacy of PAF-receptor antagonists may be due to the lack of agonist specificity of the receptor. It has been reported that LPC, oxidized phospholipids and bacterial endotoxin are all capable of activating the PAF receptor [49, 50]. In the absence of clinically useful PAF-receptor antagonists, vitamin E, as a result of its neutralizing interactions with PAF (as well as with LPC), together with its reported inhibitory effects on PAF synthesis [21], may offer a measure of protection against this pro-inflammatory PL.

There are several recent studies detailing the potential benefits of vitamin E against deteriorating lung function in the elderly, as well as in smokers, while a relationship between vitamin E intake and adult-onset asthma or wheeze has also been reported [22–27]. Although most of these studies have suggested that the protective benefit demonstrated with vitamin E is mediated predominantly *via* its antioxidant activity, it is known that in disorders such as asthma, other mediators of airway inflammation, and in particular the bioactive phospholipids, may be as important as reactive oxidants. The present study demonstrates that the bioactive phospholipids are potential mediators of airway inflammation and ciliary dysfunction and suggests that vitamin E may act as a natural antagonist of these pro-inflammatory lipids.

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