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Effect of fluticasone propionate and salmeterol on *Pseudomonas aeruginosa* infection of the respiratory mucosa *in vitro*

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Effect of fluticasone propionate and salmeterol on Pseudomonas aeruginosa *infection of the respiratory mucosa* in vitro. *R.B. Dowling, M. Johnson, P.J. Cole, R. Wilson.* ©*ERS Journals Ltd 1999.*

ABSTRACT: The purpose of this study was to investigate the effect of the corticosteroid, fluticasone propionate (FP), on *Pseudomonas aeruginosa* infection of the respiratory mucosa of an organ culture model *in vitro*.

Organ cultures infected with *P. aeruginosa* had significantly ($p \leq 0.05$) elevated levels of mucosal damage and significantly ($p \leq 0.05$) less ciliated cells compared to controls. Preincubation of tissue with FP (10^{-6} or 10^{-5} but not 10^{-7} M) prior to *P. aeruginosa* infection significantly ($p \leq 0.05$) reduced the bacterially induced mucosal damage in a concentration-dependent manner. FP (10^{-5} M) also significantly ($p \leq 0.05$) prevented loss of ciliated cells. FP did not alter the density of bacteria adherent to the different mucosal features of the organ cultures, but did reduce total bacterial numbers due to the reduced amount of damaged tissue, which is a preferred site of *P. aeruginosa* adherence.

It has previously been shown that the long-acting β_2-agonist salmeterol (4×10^{-7} M) also reduces the mucosal damage caused by *P. aeruginosa* infection, probably *via* elevation of intracellular cyclic adenosine monophosphate concentrations. Preincubation of tissue with both 10^{-7} M FP and 10^{-7} M salmeterol, concentrations at which they did not by themselves influence the effect of *P. aeruginosa* infection, significantly ($p \leq 0.05$) reduced *P. aeruginosa*-induced loss of cilia. However, there was no additional benefit from adding 4×10^{-7} M salmeterol to 10^{-6} M FP.

In conclusion fluticasone propionate reduced mucosal damage caused by *P. aeruginosa* infection *in vitro* and preserved ciliated cells. There was a synergistic action with salmeterol in the preservation of ciliated cells.

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Pseudomonas aeruginosa is an opportunistic pathogen which frequently colonizes the respiratory tract of patients with cystic fibrosis and other forms of bronchiectasis [1–3]. It is also seen as a common pathogen in the intensive care unit and sometimes in severe chronic obstructive airway disease [4]. Infection is associated with increased morbidity and mortality in cystic fibrosis and worse quality of life in bronchiectasis [1, 5, 6]. Once *P. aeruginosa* infection is established it is rarely eliminated despite vigorous antibiotic therapy. Patients sometimes require continuous antibiotic therapy in order to suppress bacterial growth and so reduce bronchial inflammation [7, 8]. However, antibiotics may be poorly tolerated when given for long periods and resistance frequently occurs. For these reasons alternative approaches to therapy are under investigation and include corticosteroids [9] and other anti-inflammatory drugs [10], mucolytics [11] and vaccination [12].

P. aeruginosa infection causes mucus hypersecretion and ulceration of the epithelium. There is a florid inflammatory response, which consists predominantly of neutrophils in the airway lumen and mononuclear cells in the bronchial wall. Bacteria adhere to mucus and areas of epithelial damage [13]. Similar observations have been made during *P. aeruginosa* infection of organ cultures [14, 15]. The mechanisms underlying *P. aeruginosa*-induced changes to the mucosa are poorly understood. *P. aeruginosa* toxins may directly injure the cell membrane [16]. Toxins may perturb cell homeostasis in other ways, for example by causing a fall in intracellular cyclic nucleotide concentrations [17]. Adenosine triphosphate (ATP) depletion leads to changes in the actin cytoskeleton, resulting in tight junction separation [18], which is commonly seen in organ cultures infected with *P. aeruginosa* [15, 19]. *P. aeruginosa* elastase also disrupts epithelial cell tight junctions [20]. Neutrophils attracted to the site of infection may also damage the epithelium [21], and inflammatory mediators released in response to infection may themselves have the potential to cause epithelial damage [22, 23].

Fluticasone propionate (FP) is a topically active novel androstane glucocorticoid [24]. It is a highly lipophilic molecule which has a high affinity for, and a rapid rate of association with, the human glucocorticoid receptor [25, 26]. Inhaled FP reduced the level of inflammatory mediators in sputum from patients with bronchiectasis [27]. Salmeterol is a potent β_2-agonist with a prolonged action [28, 29]. It has previously been shown that

salmeterol reduced falls in both intracellular cyclic adenosine monophosphate (cAMP) and ATP concentration that were associated with ciliary beat slowing in human nasal epithelial cells exposed to *P. aeruginosa* toxins [30]. Salmeterol also reduced the damage to human respiratory epithelium caused by *P. aeruginosa* infection of organ cultures, and ultrastructural damage in human nasal epithelial cells incubated with the *P. aeruginosa* toxins pyocyanin and elastase [15].

This study investigated the effect of FP on *P. aeruginosa* infection of the respiratory mucosa of an organ culture with an air/mucosa interface. In addition, the effects of FP and salmeterol together were studied to determine whether there was any additive or synergistic benefit of the combination.

Materials and methods

Bacteriology

P. aeruginosa strain P455 is a clinical isolate that has previously been studied in the authors′ laboratory [14, 15, 19, 22, 31]. It is a nonmucoid piliated strain that produces alkaline protease, elastase, phenazine pigments, lipase, deoxyribonuclease and rhamnolipid. P455 was stored at -70°C in a brain/heart infusion (BHI) broth (Oxoid, Basingstoke, UK) and glycerol (Sigma, Poole, UK) mixture (80:20) and then recovered onto BHI agar. After overnight culture, two or three colonies were dispersed in 5 mL BHI broth and incubated overnight at 37°C with agitation. The culture was diluted with BHI broth to give an optical density of 0.365 at 450 nm, which previous experiments had shown corresponded to $\sim 1.0 \times 10^8$ colony-forming units (CFU)·mL^{-1}. One millilitre of the culture was then washed twice with 10 mL phosphate-buffered saline (PBS) (Oxoid). The bacterial pellet was resuspended in 1 mL of PBS, agitated on a vortex mixer and viable counts performed.

Preparation of fluticasone propionate and salmeterol

FP (Glaxo Wellcome, Uxbridge, UK) (50 mg) was dissolved in a minimum amount of dimethyl acetamide (Sigma) and the solution made up to 10 mL with minimal essential medium (MEM) (Gibco, Paisley, UK) This solution was further diluted with MEM to yield final concentrations of 10^{-7}, 10^{-6} and 10^{-5} M. Salmeterol hydroxynaphthoate (Glaxo Wellcome) (6.03 mg) was dissolved in a minimum amount of glacial acetic acid and then diluted with PBS to give a concentration of 10^{-5} M. This was further diluted with MEM to yield final concentrations of 10^{-7} M and 4×10^{-7} M.

Organ cultures

This method has been described previously [14, 15, 19, 22, 32]. Small squares of human nasal turbinate tissue (approximately 3 mm^2 and 2–3 mm thick), were used to construct an organ culture with an air/mucosa interface.

For each experiment studying the effect of FP (n=6), eight organ cultures were prepared: control tissue incubated with MEM alone; control tissue treated with FP alone (10^{-7}, 10^{-6} or 10^{-5} M); tissue infected with *P. aeruginosa* alone; and tissue treated with FP (10^{-7}, 10^{-6} or 10^{-5} M) and then infected with *P. aeruginosa*. The relevant tissue squares were preincubated with 4 mL FP at the appropriate concentration for 2 h prior to assembly of the organ cultures. During this time, the other tissue squares were incubated with MEM alone.

For each experiment studying the effect of FP and salmeterol (n=6), nine organ cultures were prepared: control tissue incubated with MEM alone; control tissue treated with FP (10^{-6} M) and salmeterol (4×10^{-7} M) together; tissue infected with *P. aeruginosa* alone; and tissue treated with FP (10^{-7} or 10^{-6} M), salmeterol (10^{-7} or 4×10^{-7} M) or both FP and salmeterol together (10^{-7}/10^{-7} or 10^{-6} M/4×10^{-7} M) prior to infection with *P. aeruginosa*. The relevant tissue squares were preincubated with 4 mL FP for 2 h or 4 mL salmeterol for 30 min, both at the appropriate concentration, prior to construction of organ cultures. When both agents were administered together, the tissue was incubated with FP for 90 min before the addition of salmeterol.

Infection and incubation of organ cultures

Washed P. aeruginosa suspended in PBS (20 μL) were gently pipetted onto the surface of the appropriate tissue squares immediately after organ culture construction. The other tissue squares were inoculated with 20 μL PBS. All organ cultures were incubated in 5% CO_2 in a humidified atmosphere for 8h at 37°C. At the end of each experiment, the four edges of the tissue squares in organ culture were touched with a sterile loop and plated onto BHI agar in order to assess the sterility of uninfected organ cultures, and the purity of *P. aeruginosa* growth in infected organ cultures. The filter paper strip was then cut near the tissue using a sterile blade, removed with the tissue attached and fixed for scanning electron microscopy as previously described [14, 15, 32].

Assessment of tissue by means of scanning electron microscopy

At the end of each experiment, the tissue squares were given a coded number so that their original identity was unknown during analysis by scanning electron microscopy. Each tissue square was examined by the same observer using a Hitachi S-4000 scanning electron microscope (Katsuta-shi, Ibaraki-Ken, Japan). The method used to make a representative survey of the mucosal surface measuring 1.42×10^4 μm^2 has been described previously [14, 15, 19, 22, 32]. The percentage tissue surface area occupied by four mucosal features was calculated: mucus, damaged epithelium, ciliated cells and unciliated cells. Mucus was seen as either plaques of amorphous material or globular material, above the epithelial surface, or was seen being secreted from the epithelium. Extruding cells, cell debris, dead cells and loss of epithelium were scored together under the category of damaged epithelium. Unciliated areas were defined as areas not covered by cilia, with or without microvilli. The number of bacteria associated with each mucosal feature were counted. An

approximation was made when, occasionally, large numbers of bacteria were present in sheets. In these instances, observation of the tissue surrounding the bacteria enabled a judgement to be made about the mucosal feature to which they were adhering. The density of bacteria adhering to each mucosal feature was calculated in order to overcome the difficulty caused by different proportions of the organ culture surface being occupied by each mucosal feature, making comparisons between organ cultures difficult. The total number of bacteria adhering to each organ culture in the area analysed (1.42×10^4 μm^2) was also recorded.

Effect of fluticasone propionate and salmeterol on Pseudomonas aeruginosa *growth* in vitro

P. aeruginosa (P455) was retrieved onto BHI agar. After overnight culture, three colonies were dispersed in 5 mL BHI broth containing broth alone, FP (10^{-7}, 10^{-6} or 10^{-5} M), salmeterol (10^{-7} or 4×10^{-7} M) or both FP and salmeterol together (10^{-7}/10^{-7} and 10^{-6} M/4×10^{-7} M) and incubated at 37°C. Viable counts were performed hourly on these cultures over a period of 8 h by standard dilution methods.

Statistics

All values are given as mean±SEM. Comparisons of the mean percentage surface area occupied by each of the four mucosal features were analysed using the Mann–Whitney U-test. The densities of bacteria adhering to each mucosal feature and the total number of bacteria adhering to the respiratory mucosa were compared using the Wilcoxon signed-rank pair test. A p-value ≤0.05 was judged to be significant.

Results

Bacteria

The mean±SD number of *P. aeruginosa* in the 20 μL PBS used to inoculate the infected organ cultures was $6.6 \pm 0.9 \times 10^6$ CFU for the FP series of experiments and $5.0 \pm 0.3 \times 10^6$ CFU for the FP and salmeterol series of experiments. There was no significant difference between the inocula of the two series of experiments. At 8 h, all control organ cultures were sterile and all *P. aeruginosa*-infected organ cultures gave a pure growth.

Table 1. – Effect of fluticasone propionate (FP) on *Pseudomonas aeruginosa* infection of the respiratory mucosa *in vitro*

Experiment	Surface area occupied$^+$ %			
	Mucus	Damaged mucosa	Ciliated cells	Unciliated cells
Control	13.4±4.4	6.4±1.3	31.0±11.0	49.2±12.2
P. aeruginosa	8.7±4.3	55.4±7.8*	7.3±6.0*	28.6±7.2
P. aeruginosa + 10^{-7} M FP	12.3±4.4	33.6±6.1*‡§	17.8±9.1	36.3±8.4
P. aeruginosa + 10^{-6} M FP	11.3±6.7	13.7±1.7*†	19.0±9.5‡	56.0±5.6†
P. aeruginosa + 10^{-5} M FP	8.9±2.0	12.2±1.1*†	46.4±10.4†	32.5±10.0

Data are expressed as mean±SEM (n=6). $^+$: by each mucosal feature. *,†, ‡, §: p≤0.05 *versus* control, *P. aeruginosa*, *P. aeruginosa* +10^{-5} M FP, and *P. aeruginosa* +10^{-6} M FP, respectively.

Scanning electron microscopy

Control tissue had very little mucosal damage and was well ciliated (table 1). *P. aeruginosa* infection of organ cultures caused changes that have been described previously [14, 15, 19, 22]. Infection caused a significant (p≤0.05) increase in mucosal damage and decrease in the number of ciliated cells compared to control (table 1; fig. 1). Tight junctions between epithelial cells were frequently separated and bacteria adhered avidly to these areas, suggesting that this may be a route of invasion. Bacteria also adhered avidly to dead and membrane-denuded cells as well as to cellular debris. In infected organ cultures, cilia appeared disorganized, although *P. aeruginosa* rarely adhered to cilia.

FP alone (10^{-7}, 10^{-6} or 10^{-5} M) had no effect on any of the mucosal features compared to control tissue (data not

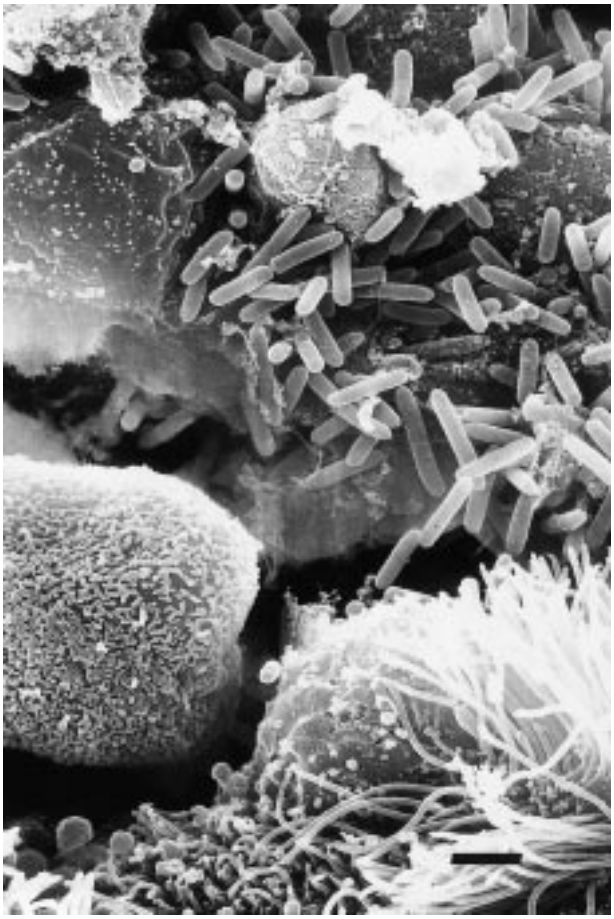

Fig. 1. – Scanning electron micrograph of human nasal turbinate tissue infected with *P. aeruginosa*. Epithelial integrity has been disrupted by the bacterial infection and *P. aeruginosa* are seen adhering to damaged epithelial cells. (Internal scale bar=0.9 μM)

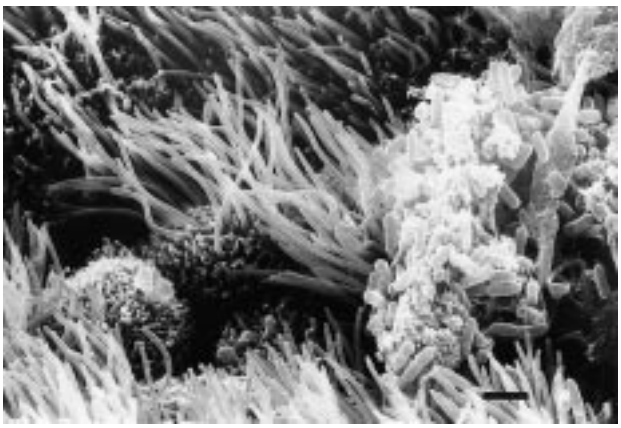

Fig. 2. – Scanning electron micrograph of human nasal turbinate tissue preincubated with FP (10^{-6}) prior to *P. aeruginosa* infection. Prior incubation with FP reduced the amount of mucosal damage caused by infection, but *P. aeruginosa* are still seen adhering to mucus above the ciliated cells. (Internal scale bar =0.9 μM)

shown). Preincubation of tissue with FP prior to *P. aeruginosa* infection reduced bacterially induced mucosal damage in a concentration-dependent fashion (table 1). FP (10^{-6} and 10^{-5} M) significantly ($p \leq 0.05$) reduced *P. aeruginosa*-induced mucosal damage (fig. 2), and the highest concentration of FP tested (10^{-5} M) significantly ($p \leq 0.05$) reduced *P. aeruginosa*-induced loss of cilia and resulted in significantly ($p \leq 0.05$) more ciliated cells compared to infected tissue preincubated with FP (10^{-6} M). The results of the lowest concentration of FP studied (10^{-7} M) did not differ significantly from those of tissue infected with *P. aeruginosa* alone. However, tissue preincubated with FP (all concentrations) prior to *P. aeruginosa* infection still had significantly ($p \leq 0.05$) elevated levels of mucosal damage compared to control, indicating that FP did not give complete protection at these concentrations.

Preincubation of tissue with either FP (10^{-6} M) or salmeterol (4×10^{-7} M) prior to *P. aeruginosa* infection significantly ($p \leq 0.05$) reduced *P. aeruginosa*-induced damage (table 2). The extent of tight junction separation appeared to be reduced. Tissue preincubated with 10^{-7} M FP or 10^{-7} M salmeterol did not significantly differ from that treated with *P. aeruginosa* alone. Tissue preincubated with both FP (10^{-6} M) and salmeterol (4×10^{-7} M) together had significantly ($p \leq 0.05$) less mucosal damage and more ciliated cells than that treated with *P. aeruginosa* alone (table 2), but the differences were similar to those seen with either agent alone. There was no synergistic or additive benefit compared to either agent alone at the same concentrations.

Preincubation of tissue with both FP and salmeterol together at the lowest concentrations used (10^{-7} M/10^{-7} M) prior to *P. aeruginosa* infection did not significantly reduce *P. aeruginosa*-induced mucosal damage, although there was a trend towards reduced damage. However, they did significantly ($p \leq 0.03$) reduce *P. aeruginosa*-induced loss of cilia (table 2). There was a synergistic effect compared to either agent alone at the same concentration: FP 2.1±1.3% (mucosal surface ciliated after 8-h infection); salmeterol 4.0±2.3%; and FP and salmeterol 26.7±11.1%.

Table 2. – Effect of fluticasone propionate (FP) and salmeterol (Salm) on *Pseudomonas aeruginosa* infection of the respiratory mucosa *in vitro*

Experiment	Surface area occupied^{+} %			
	Mucus	Damaged mucosa	Ciliated cells	Unciliated cells
Control	18.3±8.1	4.7±1.1	44.4±8.1	32.6±7.7
10^{-6} M FP+Salm 4×10^{-7} M	5.6±3.6	3.1±1.5†	26.1±13.1	65.2±15.2
P. aeruginosa	11.0±3.5	52.0±8.2*	5.2±3.1*	31.8±7.8
P. aeruginosa +10^{-7} M FP	24.9±11.4	32.3±9.0*¶	2.1±1.3*¶	40.7±12.3
P. aeruginosa +10^{-6} M FP	7.3±2.8	9.2±1.5†‡§	50.5±13.3†	33.0±13.3
P. aeruginosa +10^{-7} M Salm	19.5±7.2	38.6±5.1*‡	4.0±2.3*	37.9±9.6
P. aeruginosa +4×10^{-7} M Salm	9.2±3.4	19.1±3.7*†	30.7±12.4	41.0±12.1
P. aeruginosa +10^{-7} M/10^{-7} M FP+ Salm	13.5±5.9	27.4±8.3*	26.7±11.1$^{\dagger\S\#}$	32.4±10.9
P. aeruginosa +10^{-6} M/4×10^{-7} M FP + Salm	24.8±6.1	13.1±6.2†§	29.0±8.4$^{\dagger\S\#}$	33.1±7.0

Data are presented as mean±SEM (n=6). $^{+}$: by each mucosal feature. *,†: p:≤0.05 *versus* control and *P. aeruginosa*, respectively; $^{\ddagger, \S, \P, \#}$: p≤0.03 *versus P. aeruginosa* + 4×10^{-7} M Salm, *P. aeruginosa* +10^{-7} M Salm, *P. aeruginosa* +10^{-6} M FP, and *P. aeruginosa* +10^{-7} M FP, respectively.

Bacterial adherence to organ cultures

The interaction of *P. aeruginosa* with the organ culture was similar to that previously reported [14, 15, 19, 22]. Bacteria were seen to be most frequently associated with damaged epithelium (particularly separated tight junctions and dead cells) and mucus (fig. 1). Preincubation of tissue with FP (10^{-7}, 10^{-6} or 10^{-5}M), salmeterol (10^{-7} or 4×10^{-7} M) or both agents together (10^{-7}/10^{-7} or 10^{-6} M/4×10^{-7} M) had no effect on the density of *P. aeruginosa* adhering to each mucosal feature (tables 3 and 4). However, FP (10^{-6} but not 10^{-7} M), salmeterol (4×10^{-7} but not 10^{-7} M) or both agents together (10^{-6}/4×10^{-7} but not 10^{-7} M/10^{-7} M) significantly ($p \leq 0.03$) reduced the total number of *P. aeruginosa* adhering to the respiratory mucosa in the area surveyed (table 4). Although there was a similar trend in the FP series of experiments (table 3), the results were not significant. Neither FP or salmeterol nor both agents together at any of the concentrations analysed had any effect on *P. aeruginosa* growth *in vitro* (data not shown).

Discussion

The effect of *P. aeruginosa* infection of the respiratory mucosa in this study was similar to that previously reported [14, 15, 19, 22]. *P. aeruginosa* infection caused extensive mucosal damage, evidenced by cellular extrusion, separation of tight junctions and stripping away of the epithelium. There was preferential loss of ciliated cells. *P. aeruginosa* demonstrated a tropism for mucus and damaged epithelium, which has been reported by a number of other authors [33, 34]. It has previously been shown that

Table 3. – Effect of fluticasone propionate (FP) on the density and total number of *Pseudomonas aeruginosa* adhering to the respiratory mucosa *in vitro*

Experiment Method	Number of adherent bacteria*				
	Mucus	Damaged mucosa	Ciliated cells	Unciliated cells	Total number^{+}
P. aeruginosa	30.1±17.1	188.2±37.8	4.6±4.1	7.5±4.7	4739±1907
P. aeruginosa + 10^{-7} FP	45.1±12.4	118.0±34.0	1.6±0.8	24.1±17.4	2221±839
P. aeruginosa + 10^{-6} FP	32.7±15.2	99.9±30.8	0.3±0.3	6.3±4.5	706±203
P. aeruginosa + 10^{-5} FP	39.1±13.7	101.7±33.0	1.3±0.6	26.7±14.7	808±246

Data are expressed as mean±SEM (n=6). *: per 3.55×10^2 μm^2; $^{+}$: per 1.42×10^4 μm^2.

salmeterol reduces *P. aeruginosa*-induced damage, probably *via* a cAMP-dependent mechanism [19, 30]. A number of studies using different systems have also shown that elevation of intracellular cAMP levels may have a cytoprotective effect [35–37]. The present study demonstrates that FP reduces mucosal damage and also loss of cilia caused by *P. aeruginosa* infection in a concentration-dependent manner.

The anti-inflammatory properties of FP have been extensively documented. FP may prevent airway inflammation by downregulating the expression of pro-inflammatory cytokines, such as interleukin-8, in epithelial cells [38]. FLOWER and ROTHWELL [39] demonstrated that corticosteroids increased the synthesis of lipocortin which in turn inhibits the production of phospholipase A_2 and, hence, the production of lipid mediators such as leukotrienes, prostaglandins and platelet-activating factor. FP may also prevent tight junction separation. CASTELLINO *et al.* [40] demonstrated that dexamethasone stabilized the actin cytoskeleton of several cell types, as revealed by increased resistance of actin filaments to cytochalasin and by visible thickening of actin filament bundles. Maintenance of the epithelial permeability barrier may prevent epithelial damage since *P. aeruginosa* adhered to sites of cell separation, and bacteria adherent to the epithelial surface may increase epithelial damage due to release of toxins in the microenvironment of the epithelial surface. Corticosteroids may also exert their anti-inflammatory effect by inhibiting the formation of the inflammatory mediator nitric oxide. It has previously been demonstrated that *P. aeruginosa* infection of organ cultures leads to increased nitrite levels in the surrounding medium, which are reduced in the presence of the NO synthase (NOS) inhibitor asymmetric dimethyl arginine. *P. aeruginosa* culture filtrate also stimulated the expression of inducible NOS in an epithelial cell line *in vitro* [22].

Preincubation of tissue with FP, salmeterol or both agents together significantly ($p \leq 0.05$) reduced the total number of *P. aeruginosa* adhering to the respiratory mucosa in a concentration-dependent manner without altering the bacterial tropism for each mucosal feature (table 4). The same trend was observed for the FP series of experiments but the difference was not significant due to the large standard errors (table 3). Neither salmeterol nor fluticasone had any direct effect on *P. aeruginosa* growth at the concentrations used in these experiments. The reduction in the total number of bacteria was probably due to a reduction in the amount of damage caused by *P. aeruginosa*, to which the bacteria preferentially adhered and preservation of ciliated cells, to which they did not (tables 1 and 2). Damaged cells may also release nutrients that stimulate bacterial growth; thus, reducing the damage may also limit bacterial growth.

Tissue incubated with FP and salmeterol at the higher concentrations investigated (10^{-6} M/4×10^{-7} M) showed no added benefit with respect to mucosal damage or preservation of ciliated cells compared to either agent alone at the same concentrations (table 2). One possibility is that there is a common pathway leading to reduced epithelial damage which is maximally stimulated by either compound alone at these concentrations. Alternatively, some aspects of *P. aeruginosa*-induced damage may not be influenced by these agents. However, although tissue preincubated with the lower concentration combination of FP and salmeterol (10^{-7} M/10^{-7} M) prior to *P. aeruginosa* infection did not exhibit reduced mucosal damage, there was a trend towards reduced damage and a significant ($p \leq 0.05$) preservation of the number of ciliated cells compared to either agent alone at the same concentrations. These data would support a synergistic action between the two compounds. The β_2-adrenoceptor gene has three potential glucocorticoid response elements and

Table 4. – Effect of fluticasone propionate (FP) and salmeterol (Salm) on the density and total number of *Pseudomonas aeruginosa* adhering to the respiratory mucosa *in vitro*

Experiment Method	Number of adherent bacteria*				
	Mucus	Damaged mucosa	Ciliated cells	Unciliated cells	Total number^{+}
P. aeruginosa	36.4±17.6	113.1±26.0	1.7±1.7	1.5±0.5	2819±1083
P. aeruginosa +10^{-7} M FP	60.5±35.4	115.1±50.6	0.1±0.1	2.2±1.1	517±1481§
P. aeruginosa +10^{-6} M FP	27.6±16.2	87.3±23.3	0.5±0.5	0.6±0.2	425±128$^{\#\ddagger}$
P. aeruginosa +10^{-7} M Salm	96.6±14.1	142.3±16.2	1.1±0.7	3.4±2.6	2975±391†
P. aeruginosa +4×10^{-7} M Salm	16.4±8.0	84.7±33.6	0.7±0.5	3.4±2.3	570±173$^{\#}$
P. aeruginosa +10^{-7} M/10^{-7} M FP + Salm	30.5±15.5	101.7±26.1	0.4±0.1	1.9±0.9	1322±543
P. aeruginosa +10^{-6} M/4×10^{-7} M FP + Salm	33.1±13.2	33.7±10.8	0.5±0.2	1.6±0.7	592±184$^{\#\ddagger}$

Data are expressed as mean±SEM (n=6). *: per 3.55×10^2 μm^2; $^{+}$: per 1.42×10^4 μm^2. $^{\#}$: $p \leq 0.03$ *versus P. aeruginosa*; †: $p \leq 0.01$ *versus P. aeruginosa* + 4×10^{-7} M Salm; ‡: $p \leq 0.03$ *versus P. aeruginosa* + 10^{-7} M Salm; §: $p \leq 0.05$ *versus P. aeruginosa* + 10^{-6} M FP.

steroids may increase the expression and stimulation of β_2-adrenoceptors by increasing their rate of transcription [41, 42]. In addition, the efficacy of coupling between the β_2-adrenoceptor and the stimulating G proteins, which mediate stimulation of adenylate cyclase, has also been reported to be modified *via* glucocorticoids [43]. However, whether this transcriptional control of β_2-adrenoceptors occurred in the organ culture over 8 h remains to be determined.

It has previously been shown that agents that elevate intracellular cyclic adenosine monophosphate levels protect the respiratory epithelium against *Pseudomonas aeruginosa*-induced damage [15, 19]. The present study demonstrates that fluticasone propionate also reduces the epithelial damage caused by *Pseudomonas aeruginosa* infection. Fluticasone propionate and salmeterol, when administered together at lower concentrations, exhibited a synergistic effect with respect to the preservation of ciliated cells. This result may have clinical significance as it is thought that ciliated cells are the most sensitive to damage by bacterial infection [14, 44]. In bronchiectasis, the condition of the airways and bacterial resistance to antibiotics makes eradication of *Pseudomonas aeruginosa* infection by means of antibiotic treatment impossible. Fluticasone propionate and salmeterol or their combination could augment antibiotic treatment by maintaining epithelial integrity and reducing the number of sites available for bacterial adherence. Bacteria adherent to mucus are cleared *via* the mucociliary system, which would be enhanced by the preservation of ciliated cells. However, further work is required to determine whether these *in vitro* observations translate into a clinically meaningful effect.

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