Eur Respir J 2000; 15: 68–74
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European Respiratory Journal
ISSN 0903-1936

Budesonide and formoterol inhibit ICAM-1 and VCAM-1 expression of human lung fibroblasts

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ABSTRACT: The glucocorticoid budesonide and the long-acting β_2-adrenoceptor agonist formoterol are used in asthma therapy for their anti-inflammatory and bronchodilating effects, respectively. Since expression of adhesion molecules on resident cells in the lung plays an important role in asthmatic inflammatory responses, the effects of these drugs on the cytokine-induced intercellular adhesion molecule-1 (ICAM)-1 and vascular cell adhesion molecule-1 (VCAM)-1 expression of human lung fibroblasts were investigated.

Budesonide and formoterol were added in the absence or presence of interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), interferon gamma (IFN-γ) or IL-4 to human lung fibroblasts; ICAM-1 and VCAM-1 expression were measured after 8 h using a cell surface enzyme linked immunosorbent assay (ELISA).

It was found that both budesonide and formoterol significantly inhibited ($p<0.05$) the increased expression of ICAM-1 and VCAM-1 after stimulation with IL-1β (maximal inhibition (median (25–75% percentiles) 50 (48–52) and 61% (42–69), respectively, with budesonide and 55 (50–73) and 86% (64–94), respectively, with formoterol (10^{-7} M)), TNF-α (maximal inhibition 49 (46–57) and 57% (44–68), respectively, with budesonide and 44 (40–75) and 62% (52–83) respectively, with formoterol), IFN-γ (maximal inhibition 64% (41–67) with budesonide and 39% (29–49) with formoterol for ICAM-1) and IL-4 (maximal inhibition 82% (69–92) with budesonide and 43% (33–67) with formoterol for VCAM-1) in a dose-dependent manner.

The results show that budesonide, as well as formoterol, in probably clinically relevant concentrations inhibits cytokine-induced adhesion molecule expression on human lung fibroblasts from a concentration of 10^{-9} M. This inhibitory effect on resident cells may have implications for the infiltration of inflammatory cells into pulmonary tissue during therapy with these drugs in asthma.

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Keywords: Adhesion molecules
budesonide
formoterol
lung fibroblasts

Received: May 4 1999
Accepted after revision September 28 1999

This study was supported by a grant Astra Zeneca, Zoetermeer, the Netherlands.

Glucocorticoids and β_2-agonists are currently used extensively in the treatment of asthma. The glucocorticoid budesonide is used for its anti-inflammatory properties, resulting, for instance, in a reduced number of eosinophils in sputum and in peripheral blood after allergen challenge [1]. A main target of glucocorticoid action is inhibition of inflammatory cell activation, demonstrated for T-cell proliferation [2], blood mononuclear cell cytokine production [3] and eosinophil chemokine production and survival [4, 5]. More recently, it has become apparent that glucocorticoids also exhibit inhibitory effects on the activation of resident cells in the lung. Budesonide, as well as dexamethasone, is able to inhibit the cytokine production and adhesion molecule expression of endothelial and epithelial cells [6, 7], and the cytokine production of smooth muscle cells [8] and fibroblasts [9].

The β_2-agonist formoterol, mainly used in asthma therapy for its long-acting bronchodilating effect, can also inhibit allergic inflammation, probably by increasing intracellular cyclic adenosine monophosphate (cAMP) levels [10]. The inhibitory effects of long-acting β_2-agonists on eosinophils and other inflammatory cells have been described [11, 12]. Some *in vivo* studies in humans show inhibitory effects of formoterol on the late asthmatic response (LAR), but do not show decreased eosinophil numbers in blood and sputum [13, 14]. However, WALLIN *et al.* [15] found a reduced number of submucosal eosinophils in patients with more severe airway inflammation after treatment with inhaled formoterol. In guinea-pigs, formoterol inhibits the LAR as well as the eosinophilic influx [16]. In addition, formoterol is able to inhibit granulocyte adhesion to vascular endothelium *in vitro* [17].

The infiltration of activated eosinophils into bronchial as well as peripheral lung tissue, along with other inflammatory cells [18, 19], is dependent on chemotactic stimuli and adhesion phenomena. Fibroblasts, resident cells of the connective tissue, are able to secrete a variety of inflammatory cytokines and chemokines [20, 21] as well as expressing intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These adhesion molecules are upregulated after cytokine exposure, with differential roles for interferon gamma (IFN-γ),

selectively upregulating ICAM-1 expression, and interleukin (IL)-4, selectively upregulating VCAM-1 expression [22, 23]. They both have been demonstrated to play a role in polymorphonuclear leukocyte transmigration over fibroblasts [24] and T-cell adhesion to fibroblasts *in vitro* [25]. Eosinophils have been shown at least to adhere to fibroblasts *via* an ICAM-1-dependent mechanism [26], but are known to express very late activation antigen-4, which adheres to fibronectin and VCAM-1 and could be responsible for the selective eosinophil infiltration into tissue compared to that of neutrophils [27]. Thus, lung fibroblasts may be important regulators of inflammatory cell infiltration in asthma.

The beneficial effects of budesonide and formoterol on clinical parameters in asthma can partly be attributed to modulation of adhesion molecule expression on resident lung tissue cells. In the present study, the effect of the glucocorticoid budesonide and the β_2-agonist formoterol on ICAM-1 and VCAM-1 expression on human lung fibroblasts *in vitro*, as induced by the inflammatory cytokines IL-1β, tumour necrosis factor-α (TNF-α), IFN-γ or IL-4, was investigated.

Materials and methods

Fibroblast culture

Pulmonary parenchyma was obtained from bilobar lung resection material (part of healthy lobe) after oncological surgery in a nonasthmatic individual. The tissue was cultured using the explant technique. Fibroblasts were cultured in Ham′s F12 medium (BioWhittaker Verviers, Belgium), supplemented with 10% foetal calf serum (Bodinko, Alkmaar, the Netherlands), 125 U·mL^{-1} sodium penicillin G (Yamanouchi Pharma, Leiderdorp, the Netherlands) and 125 µg·mL^{-1} streptomycin sulphate (RadiumEarma-Fisiopharma, Milan, Italy), hereafter referred to as Ham′s complete medium. Fibroblast passage was performed by means of trypsinization with trypsin/ethylenediamine tetra acetic acid (BioWhittaker). Passage was carried out in a 1:4 ratio, the cells grown to confluence (passage 5) in 6–7 days microscopic examination) in 96-well culture plates (Costar Europe, Badhoevedorp, the Netherlands) and used for experiments. Fibroblast characterization was performed with antibodies directed against vimentin, cytokeratin, desmin, smooth muscle actin and fibronectin using fluorescence microscopy. Fibroblast purity was >98%, the only contaminating cells being smooth muscle cells. As a control for heterogeneity of fibroblasts derived from different donor tissue, fibroblast cultures derived from the pulmonary parenchyma of three other donors were also assessed: comparable inhibitory effects of the drugs were seen.

Drugs

Budesonide was obtained from a Pulmicort Turbuhaler (Astra Pharmaceutica, Zoetermeer, the Netherlands) and dissolved in 96% ethanol at a concentration of 10^{-2} M. Subsequently, solutions of 10^{-10}–10^{-7} M budesonide were prepared in Ham′s complete medium. Formoterol fumarate dihydrate (Astra Draco, Lund, Sweden) was dissolved in dimethyl sulphoxide at a concentration of 10^{-2} M. Working solutions (10^{-10}–10^{-7} M) were prepared in Ham′s complete medium. The viability of confluent fibroblasts after incubation with different concentrations of budesonide and formoterol was assessed using trypan blue exclusion and was always >95%.

Incubations

Confluent fibroblast layers in 96-well plates were preincubated for 30 min with different concentrations of budesonide or formoterol, followed by an 8-h stimulation with 1 U·mL^{-1} IL-1β (Boehringer Mannheim, Mannheim, Germany), 100 U·mL^{-1} TNF-α (Genzyme, Cambridge, MA, USA), 2 U·mL^{-1} IFN-γ, 5 ng·mL^{-1} IL-4 (Boehringer Mannheim) or Ham′s complete medium (control) in the presence of the same concentration of drug (total volume 100 µL). In a previous study, it was shown that IL-1β and TNF-α were able to induce both ICAM-1 and VCAM-1 upregulation, whereas IFN-γ specifically induced ICAM-1 and IL-4 specifically induced VCAM-1 upregulation [23]; maximal expression was reached after approximately 8 h of stimulation. Vehicle controls were assessed for drug concentrations of 10^{-8} M and 10^{-7} M and did not influence ICAM-1 or VCAM-1 upregulation (data not shown). After incubation with cytokines, fibroblasts were washed twice with cold phosphate-buffered saline (PBS) supplemented with 0.01% $CaCl_2$, and fixed for 10 min in 96% ethanol at 4°C. Fibroblasts were dried on air for 30 min and stored at 4°C for a maximum of 14 days until determination of adhesion molecule expression.

Cell surface enzyme-linked immunosorbent assay

Determination of ICAM-1 and VCAM-1 expression was performed using a modified cell surface enzyme-linked immunosorbent assay according to Piela Smith *et al.* [28]. Briefly, fibroblast layers were incubated with 1% bovine serum albumin (Merck, Darrnstadt, Germany) in PBS for 1 h to block nonspecific binding sites. The cells were incubated with anti-ICAM-1 (2 µg·mL^{-1}), anti-VCAM-1 (10 µg·mL^{-1}) (R&D Systems, Abingdon, UK) or immunoglobulin G1 (IgG1) isotype control (4 µg·mL^{-1}) (Centraal Laboratorium van de Bloedtransfusiedienst, Amsterdam, the Netherlands) for 120 min at room temperature (20°C), washed according to a five-step washing procedure performed by a Microplate Strip Washer (Bio-Tek Instruments, Burlington, NJ, USA) and incubated with 50 µL horseradish peroxidase-conjugated rabbit anti-mouse antibody (DAKO, Glostrup, Denmark; 1:500) for 30 min. After the second washing procedure, 200 µL substrate solution containing 1.25 mg·mL^{-1} *o*-phenylene diamine dihydrochloride (Sigma Chemical CO, St Louis, MO, USA) was added and colour development was stopped after 30 min by the addition of 50 µL 3 M H_2SO_4. The absorbance (optical density (OD)) at 490 nm was measured using a microplate reader supported by SoftmaxPro software (Molecular Devices, Sunnyvale, CA, USA).

Data analysis

ICAM-1 and VCAM-1 expressions were calculated from the mean OD (at 490 nm) of quadruplicate determinations within one experiment after subtraction of the OD of the

IgG1 isotype control. Coefficients of variation were not >10%; outlying ODs within quadruplicates were only left out when they exceeded 2SD of the mean. At least five separate experiments for each cytokine stimulation were performed. To evaluate the dose-dependent effects of budesonide and formoterol, the mean ODs of the quadruplicate determinations representing ICAM-1 and VCAM-1 expression were analysed using the Friedman test. To further evaluate statistical differences between samples, the nonparametric Wilcoxon signed-rank test for related samples was used. Differences were considered significant at $p<0.05$. Percentages of inhibition are presented as medians with 25–75% percentiles in parentheses.

Results

Effects of budesonide

Budesonide and formoterol both inhibited all cytokine-induced upregulation of ICAM-1 and VCAM-1 on human lung fibroblasts. IL-1β upregulated ICAM-1 and VCAM-1 expression after 8 h of stimulation by 708 and 213% compared to baseline, respectively.

Budesonide at a concentration of 10^{-10}–10^{-7} M significantly inhibited the IL-1β-induced ICAM-1 upregulation (fig. 1a); maximal inhibition was 50% [48–52] at 10^{-7} M. A higher concentration was needed to significantly inhibit the IL-1β-induced VCAM-1 upregulation, *i.e.* 10^{-9}–10^{-7} M budesonide (fig. 1a); maximal inhibition was 61% (42–69) at 10^{-7} M. The TNF-α-induced ICAM-1 and VCAM-1 upregulation (758 and 263% of baseline, respectively) (fig. 2a), as well as IFN-γ induced ICAM-1 upregulation (586% of baseline) (fig. 3), were significantly inhibited at $\geq 10^{-9}$ M, whereas IL-4-induced VCAM-1 upregulation (252% of baseline) was significantly inhibited at $\geq 10^{-10}$ M (fig. 4). TNF-α-stimulated ICAM-1 upregulation was maximally inhibited by 49% (46–57) and VCAM-1 upregulation by 57% (44–68) at 10^{-7} M. IFN-γ-induced ICAM-1 upregulation was maximally inhibited by 64% (41–67) and IL-4-induced VCAM-1 upregulation by 82% (69–92) at 10^{-7} M. The effect on unstimulated fibroblasts was also assessed: basal VCAM-1 expression was significantly reduced by 10^{-9} and 10^{-7} M budesonide (maximal reduction to 89% (79–95) of baseline at 10^{-7} M), whereas basal ICAM-1 expression was only significantly reduced by 10^{-9} M (reduction to 62% (47–99) of baseline) (data not shown).

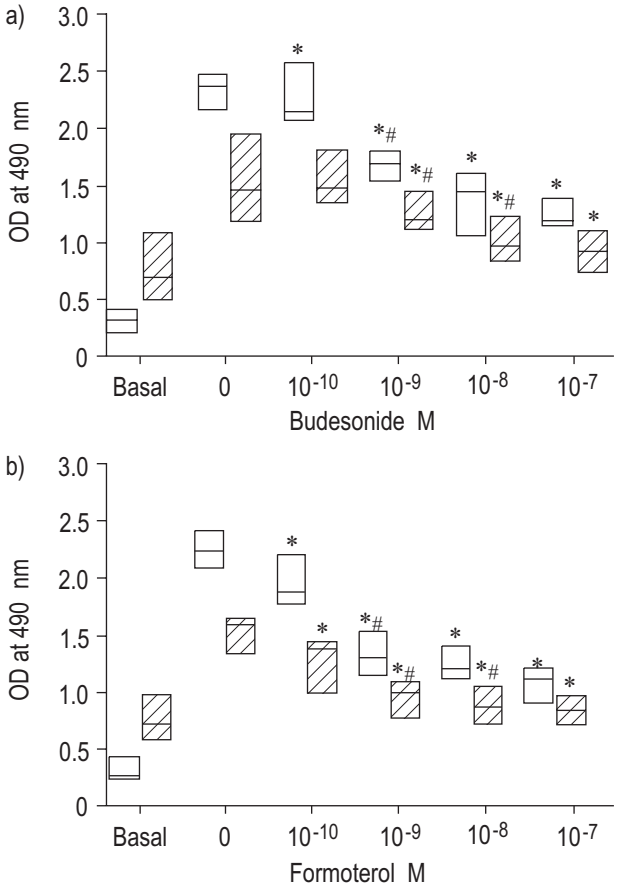

Fig. 1. – Effect of: a) budesonide at 10^{-10}–10^{-7} M (n=7); and b) formoterol at 10^{-10}–10^{-7} M (n=5) on the interleukin (IL)-1β-induced intercellular adhesion molecule-1 (□) and vascular cell adhesion molecule-1 (▨) expression of human lung fibroblasts. Data are presented as median and 25–75% percentiles after subtraction of isotype control. OD: optical density. *: $p<0.05$ *versus* IL-1β alone; #: $p<0.05$ *versus* one drug concentration step lower (Wilcoxon signed-rank test).

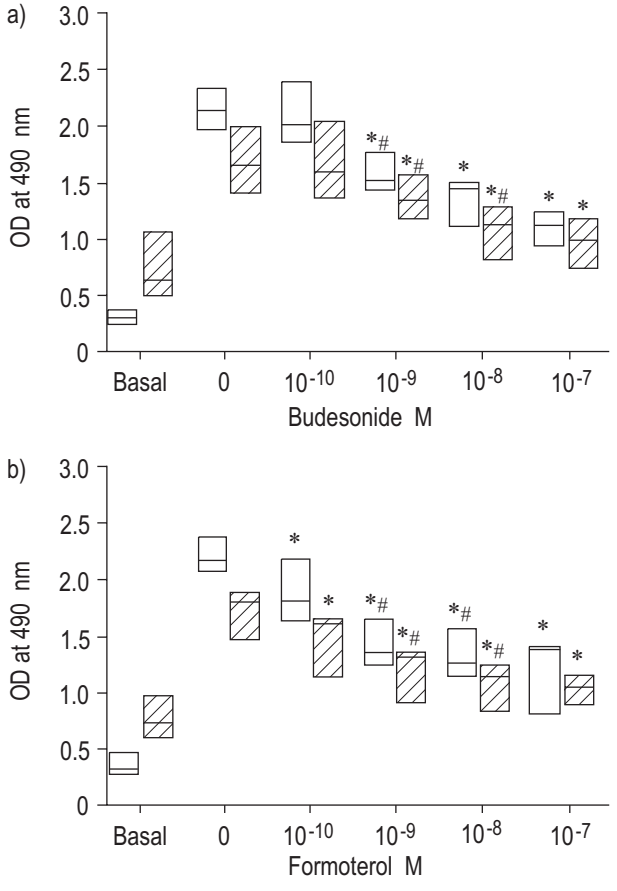

Fig. 2. – Effect of: a) budesonide at 10^{-10}–10^{-7} M (n=7); and b) formoterol at 10^{-10}–10^{-7} M (n=5) on the tumour necrosis factor-α (TNF-α)-nduced intercellular adhesion molecule-1 (□) and vascular cell adhesion molecule-1 (▨) expression of human lung fibroblasts. Data are presented as median and 25–75% percentiles after subtraction of isotype control. OD: optical density. *: $p<0.05$ *versus* TNF-α alone; #: $p<0.05$ *versus* one drug concentration step lower (Wilcoxon signed-rank test).

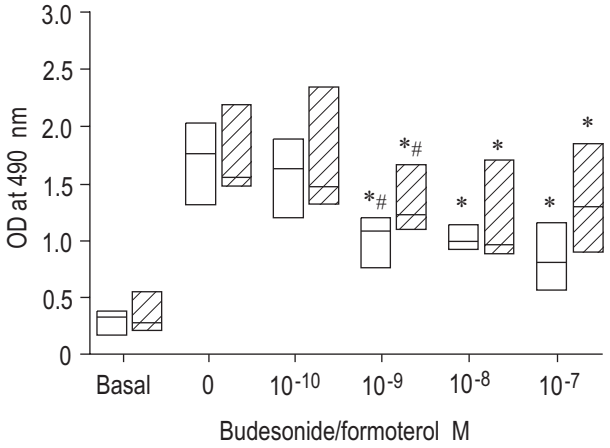

Fig. 3. – Effect of: a) budesonide at 10^{-10}–10^{-7} M (□ n=7) and formoterol at 10^{-10}–10^{-7} M (▨ n=5) on the interferon gamma (IFN-γ)-induced intercellular adhesion molecule-1 expression of human lung fibroblasts. Data are presented as median and 25–75% percentiles after subtraction of isotype control. OD: optical density. *: p<0.05 *versus* IFN-γ alone; #: p<0.05 *versus* one drug concentration step lower (Wilcoxon signed-rank test).

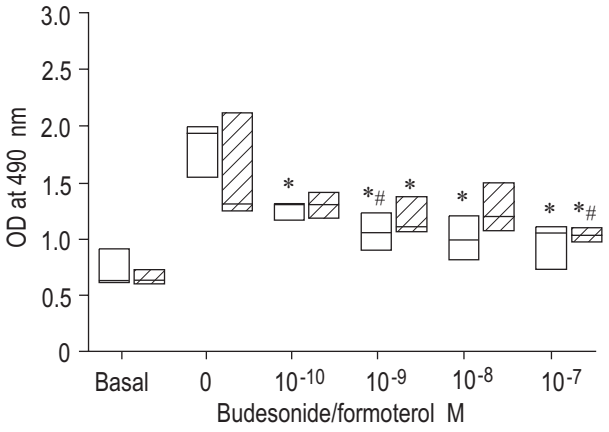

Fig. 4. – Effect of budesonide at 10^{-10}–10^{-7} M (□ n=5) and formoterol at 10^{-10}–10^{-7} M (▨ n=7) on the interleukin (IL)-4-induced vascular cell adhesion molecule-1 expression of human lung fibroblasts. Data are presented as median and 25–75% percentiles after subtraction of isotype control. OD: optical density. *: p<0.05 *versus* IL-4 alone; #: p<0.05 *versus* one drug concentration step lower (Wilcoxon signed-rank test).

Dose-dependent inhibition by budesonide was found for all cytokine stimulations (p<0.05, Friedman test). Between samples significant differences in the extent of inhibition were found between 10^{-10} and 10^{-9} M budesonide for the IL-1β- and TNF-α-induced ICAM-1 upregulation, and between 10^{-9} and 10^{-8} M for the IL-1β- and TNF-α-induced ICAM-1 and VCAM-1 upregulation (figs. 1a and 2a). For IFN- (fig. 3) and IL-4 (fig. 4) stimulation, there was a significant difference in inhibition of ICAM-1 and VCAM-1 upregulation, respectively, between 10^{-10} and 10^{-9} M. This indicates dose-dependent inhibition by budesonide up to 10^{-8} M for IL-1β and TNF-α-induced VCAM-1 upregulation and up to 10^{-9} M for IL-1β-, TNF-α, IFN-γ-induced ICAM-1 and IL4-induced VCAM-1 upregulation. At 10^{-8} M budesonide, IL-1β- and TNF-α- induced upregulation of VCAM-1 was significantly more strongly inhibited (p<0.05) than the upregulation of ICAM-1.

Effects of formoterol

Formoterol significantly inhibited (p<0.05) ICAM-1 and VCAM-1 upregulation (1061 and 237% compared to baseline, respectively) induced by IL-1β stimulation at a concentration of 10^{-10}–10^{-7} M; maximal inhibition was 55% (50–73) at 10^{-7} M and 86% (64–94) at 10^{-8} M (fig. 1b). TNF-α-induced ICAM-1 and VCAM-1 upregulation (828 and 253% of baseline, respectively) were also significantly inhibited (p<0.05) at a concentration of 10^{-10}–10^{-7} M; maximal inhibition was 44% (40–75) and 62% (52–83), respectively, at 10^{-7} M (fig. 2b). The IFN-γ-induced ICAM-1 upregulation (538% of baseline) was inhibited at ≥10^{-9} M; maximal inhibition was 39% (29–49) at 10^{-8} M (fig. 3), whereas, the IL-4-induced VCAM-1 upregulation (241% of baseline) was significantly inhibited by 10^{-9} and 10^{-7} M formoterol; maximal inhibition was 43% (33–67) at 10^{-7} M (fig. 4). Basal expression of ICAM-1 and VCAM-1 on unstimulated fibroblasts was also significantly reduced by 10^{-9}–10^{-7} M formoterol; maximal reduction was to 57 (51–67) and 84% (76–88) of baseline, respectively, at 10^{-7} M.

There was a dose-response relationship for the inhibition by formoterol of all cytokine stimulations (p<0.05, Friedman test). Between samples, significant differences were observed in the extent of inhibition by formoterol between 10^{-10} and 10^{-9} M for the IL-1β- (fig. 1b) as well as the TNF-α-induced (fig. 2b) ICAM-1 and VCAM-1 upregulation. There were also significant differences between the extent of inhibition by 10^{-9} and 10^{-8} M formoterol of the TNF-a-induced ICAM-1 upregulation and of the IL-1β- and TNF-α-induced VCAM-1 upregulation. For IFN-γ stimulation, there was a significant difference in inhibition by formoterol of ICAM-1 upregulation between 10^{-10} and 10^{-9} M (fig. 3). For IL-4 stimulation, there was a significant difference in inhibition of VCAM-1 upregulation between 10^{-7} and 10^{-8} M (fig. 4). This indicates a dose-dependent inhibition by formoterol up to 10^{-8} M for IL-1β-induced VCAM-1 and TNF-α-induced ICAM-1 and VCAM-1 upregulation and up to 10^{-9} M for IL-1β- and IFN-g-induced ICAM-1 upregulation. All concentrations of formoterol inhibited VCAM-1 upregulation significantly more strongly than ICAM-1 upregulation after IL-1β and TNF-α stimulation (table 1). Formoterol displayed stronger inhibition than budesonide of VCAM-1 upregulation, especially after IL-1β stimulation (table 1). In contrast, budesonide tended to be stronger than formoterol in inhibiting ICAM-1 or VCAM-1 upregulation after IFN-γ and IL-4 stimulation, respectively.

Discussion

ICAM-1 and VCAM-1 are important adhesion molecules in asthma with regard to infiltration of inflammatory cells into the lung. The present study shows that the glucocorticoid budesonide and the long-acting β_2-agonist formoterol both inhibit IL-1β-, TNF-α-, IFN-γ- and IL-4-induced ICAM-1 and VCAM-1 upregulation on human lung fibroblasts. Moreover, a small reduction in basal ICAM-1 and VCAM-1 levels by both drugs was also found.

Table 1. – Inhibitory effects of budesonide and formoterol on the intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression of human lung fibroblasts stimulated with interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), interferon gamma (IFN-γ) or IL-4

	Drug concentration M	Budesonide	Formoterol
ICAM-1/IL-1β	10^{-10}	7 (2–14)	10 (9–21)
	10^{-9}	33 (26–43)	47 (41–53)*
	10^{-8}	48 (37–56)	49 (47–56)
	10^{-7}	50 (48–52)	55 (50–73)
VCAM-1/IL-1β	10^{-10}	-11 (-19–4)$^{\#}$	29 (22–50)*$^{\#}$
	10^{-9}	38 (13–50)	72 (61–86)*,$^{\#}$
	10^{-8}	57 (50–75)$^{\#}$	86 (64–94)*,$^{\#}$
	10^{-7}	61 (42–69)	80 (69–105)$^{\#}$
ICAM-1/TNF-α	10^{-10}	-4 (3–14)	13 (9–27)
	10^{-9}	36 (28–36)	43 (34–47)
	10^{-8}	42 (33–54)	45 (37–54)
	10^{-7}	49 (46–57)	44 (40–75)
VCAM-1/TNF-α	10^{-10}	-3 (22–5)$^{\#}$	28 (15–39)*,$^{\#}$
	10^{-9}	30 (23–44)	52 (46–63)$^{\#}$
	10^{-8}	56 (44–69)$^{\#}$	62 (59–71)$^{\#}$
	10^{-7}	57 (44–68)	62 (52–83)$^{\#}$
ICAM-1/IFN-γ	10^{-10}	6 (-5–17)	11 (-14–15)
	10^{-9}	53 (38–58)	31 (23–34)
	10^{-8}	58 (45–66)	39 (29–49)
	10^{-7}	64 (41–67)	22 (9–50)
VCAM-1/IL-4	10^{-10}	26 (17–63)	12 (1–45)
	10^{-9}	73 (47–84)	34 (11–58)
	10^{-8}	80 (66–81)	31 (-3–48)
	10^{-7}	82 (69–92)	43 (33–67)

Data are presented as median (25–75% percentiles). *: $p<0.05$ compared to inhibition by budesonide; $^{\#}$: $p<0.05$ compared to inhibition of ICAM-1 expression.

Concentrations of budesonide 10^{-10}–10^{-8} M have been considered to be of clinical relevance, since *in vivo* concentrations range from 10^{-9} M in blood to 10^{-8} M in lung tissue, 90 min after a single inhaled dose of 1,600 μg [29]. This is the first study showing that budesonide in clinically relevant doses inhibits ICAM-1 and VCAM-1 expression on human lung fibroblasts in a dose-dependent way in all cytokine-stimulated conditions. This finding is compatible with similar results obtained using an epithelial cell line in which budesonide (10^{-8} inhibited the basal expression and the IFN-γ-induced upregulation of ICAM-1 [6]. The observed *in vitro* inhibition of inflammatory cytokine-induced adhesion molecule expression by budesonide on human lung fibroblasts is in agreement with the reduced eosinophil influx observed in asthmatic patients after budesonide treatment [1]. The underlying mechanisms of the inhibitory activity of glucocorticoids have been extensively described. Glucocorticoid bound to the glucocorticoid receptor can interact with various transcription factors, preventing attachment of these factors to their binding sequences on the promoters of several genes. These transcription factors are necessary for the induction of several messenger ribonucleic acids by cytokine.stimulation. Glucocorticoids can also inhibit the transcription factor nuclear factor-κB (NFκB) by inducing expression of its inhibitory factor inhibitory-κB [30]. The ICAM-1 and VCAM-1 gene both contain activating protein-1 (AP-1) and NFκB binding sites, providing modulatory sites for glucocorticoids [31–34].

To the authors′ knowledge, this is the first report of the inhibitory action of the β_2-agonist formoterol on ICAM-1 and VCAM-1 upregulation on resident lung cells, such as fibroblasts. Formoterol dose-dependently inhibited the IL-1β-, TNF-α-, IFN-γ- and IL-4-induced upregulation of ICAM-1 and/or VCAM-1 on human lung fibroblasts. In support of these results, several studies have shown inhibition of granulocyte adhesion to endothelium or epithelium by other long-acting β_2-agonists [17, 35]. This phenomenon could not be fully explained in these studies, although it was thought to be primarily an effect on the endothelial or epithelial cells. It is thus very likely that the antiadhesive effect of β_2-agonists in those studies was caused by modulation of adhesion molecule expression on endothelial and epithelial cells, as shown for fibroblasts in the present study. β_2-agonists can act *via* binding to β_2-adrenoceptors, which are also present on fibroblasts [36]. Downregulation of ICAM-1 expression by the short-acting β_2-agonist fenoterol has been described in epithelial cells [37]. Signalling by β_2-adrenoceptors is *via* the adenylate cyclase pathway, increasing intracellular cAMP and eventually activating cAMP-responsive element binding protein (CREB) [10]. The activated CREB is able to interact with transcription factors, such as AP-1, thereby inhibiting gene transcription [38]. Another study has shown direct cAMP involvement in cytokine-induced ICAM-1 and VCAM-1 upregulation in smooth muscle cells [39]. Therefore, it is possible that formoterol also modulates adhesion molecule upregulation at this level on fibroblasts. Formoterol has been detected in plasma samples at a concentration of 2.5×10^{-10} M 1 h after inhalation of a 120-μg dose [40], which is six times higher than a conventional dose. No results are available regarding concentrations reached in lung tissue, but locally higher concentrations may very well be present after regular inhaled doses. In guinea-pig models, eosinophil influx into the lungs after challenge was inhibited by formoterol [11, 16]. In humans, there is also evidence for direct inhibitory effects on mast cell and eosinophil infiltration *in vivo* after inhaled formoterol therapy [15]. This could be due, in part, to decreased expression of adhesion molecules by lung fibroblasts, thus contributing to the reduction in asthmatic inflammation.

VCAM- 1 upregulation was more strongly inhibited by formoterol than ICAM-1 upregulation after IL-1β and TNF-α stimulation and to a lesser extent by budesonide under these particular experimental conditions. Formoterol tended to inhibit VCAM-1 upregulation more strongly than did budesonide after IL-1β and TNF-α stimulation, whereas budesonide more strongly inhibited IFN-γ-induced ICAM-1 and IL-4- induced VCAM-1 upregulation. This may be explained by differences in the molecular mechanisms of the drugs, as well as different signal transduction pathways of the cytokines. Overall, budesonide and formoterol were both able to inhibit ICAM-1 and VCAM-1 upregulation, which effect was not dependent on the type of cytokine stimulus used in this study. It would be interesting to know the combined effect of these drugs, since they are often used in asthma therapy simultaneously, and especially because cAMP pathways are able to interact with glucocorticoid receptor pathways at different levels

[41, 42]. This issue is currently under investigation in the authors′ laboratory.

In conclusion, both budesonide (at therapeutically relevant doses) and formoterol (possibly at therapeutically relevant doses) inhibit interleukin-1β-, tumour necrosis factor-α-, interferon gamma- and interleukin-4 induced up-regulation of the adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 on human lung fibroblasts. The authors hypothesize that the beneficial effects of inhaled budesonide and inhaled formoterol in asthma could be due, in part, to modulation of the expression of adhesion molecules on resident cells in the lung. tissue, thus diminishing infiltration and/or retention of inflammatory cells.

Acknowledgements. The authors thank M. Boorsma for critically reading the manuscript.

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