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Budesonide reduces vascular endothelial growth factor secretion and expression in airway (Calu-1) and alveolar (A549) epithelial cells

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Abstract

Vascular endothelial growth factor (VEGF), a cytokine expressed in the respiratory epithelial cells, induces vascular hyperpermeability and edema, symptoms that are alleviated by budesonide, an anti-asthma corticosteroid. However, modulation of VEGF levels by budesonide in the respiratory epithelium has not been studied. In this study, we investigated the mechanisms of VEGF secretion using brefeldin A and monensin in human airway (Calu-1) and alveolar (A549) epithelial cells, and further determined whether budesonide inhibits VEGF secretion and mRNA expression through a glucocorticoid receptor-mediated mechanism. In both cell types, VEGF secretion was inhibited by brefeldin A and monensin, suggesting vesicular transport of VEGF through endoplasmic reticulum (ER)–golgi pathway. At concentrations devoid of cytotoxicity, budesonide reduced VEGF secretion and VEGF mRNA expression in both cell types and these effects were inhibited by mifepristone (RU 486), a glucocorticoid receptor antagonist, suggesting that budesonide reduces VEGF secretion and expression through its glucocorticoid receptor-mediated action. Also, budesonide-mediated inhibition of VEGF mRNA was time- and protein synthesis-dependent. Thus, budesonide may be of potential value in treating disorders of the respiratory tract that are associated with VEGF elevation.

Keywords: VEGF (vascular endothelial growth factor); Budesonide; Respiratory; Epithelial; Calu-1 cell; A549 cell

1. Introduction

Vascular hyperpermeability is often mediated by vascular endothelial growth factor (VEGF) or vascular permeability factor (VPF), an endothelial specific mitogen that is abundant in the lung (Berse et al., 1992). Within the lung, VEGF is distributed in the epithelial cells of airways and alveoli and also in alveolar macrophages (Maniscalco et al., 1995; Tolnay et al., 1998). Through its ability to induce vascular hyperpermeability, edema, and angiogenesis (Dvorak et al., 1999; Kaner et al., 2000), VEGF is proposed to play a key role in the pathogenesis of inflammatory respiratory disorders such as pulmonary hypertension (Christou et al., 1998), inflammatory airway diseases (McColley et al., 2000), and nasal polyps (Coste et al., 2000), disorders of the alveolar, airway, and nasal epithelia, respectively. As these disorders are characterized by tissue remodeling via angiogenesis, vascular hyperpermeability, and edema, and VEGF plays a pivotal role in all these events, inhibition of VEGF appears to be a novel strategy in alleviating these disorders.

Corticosteroids are the drugs of choice in the treatment of inflammatory disorders of the respiratory tract such as asthma (Barnes, 1998). Budesonide, a potent anti-asthma corticosteroid with high receptor affinity, airway selectivity, and prolonged tissue retention (Brogden and McTavish, 1992), inhibits inflammatory symptoms such as edema and vascular hyperpermeability (Erlansson et al., 1989; Svensjo, 1990). The anti-inflammatory mechanism of budesonide entails inhibition of transcription factors such as activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB) by trans-repression, thereby decreasing the expression of several genes encoding inflammatory mediators such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α) in inflammatory as well as respiratory epithelial cells (Adcock et al., 1999). This inhibition of the cytokines results in the stabilization of the endothelial cell layer (Boschetto et al., 1991). In addition, inflammatory cells as well as epithelial cells in the respiratory tract release VEGF (Coste et al., 2000; Vento et al., 2000). However, the effect of budesonide on VEGF secretion from these cells is not known.

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As VEGF is known to induce microvascular hyperpermeability and edema (Dvorak et al., 1999; Kaner et al., 2000), and because the inhibitory effect of budesonide on these symptoms is well documented (Erlansson et al., 1989; Svensjo, 1990), we hypothesized that budesonide can inhibit VEGF secretion and expression in respiratory epithelial cells. To this end, we assessed the mechanisms of VEGF secretion and the effects of budesonide treatment on VEGF secretion, VEGF mRNA expression, and cytotoxicity in Calu-1 and A549 cells, cells that represent the airway and alveolar epithelia, respectively. In addition, the role of glucocorticoid receptor in budesonide-mediated effects on VEGF was evaluated. This is the first study to report the effects of budesonide on VEGF secretion and expression in any cell model.

2. Materials and methods

2.1. Chemicals

Cell culture materials and reagents were obtained from Gibco (Grand Island, NY) and Becton Dickinson Labware (Franklin Lakes, NJ). Brefeldin A, budesonide, cycloheximide, mifepristone (RU 486), monensin, and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Triazolyl blue) were obtained from Sigma (St. Louis, MO). All chemicals were of the highest purity available commercially.

2.2. Cell culture

Calu-1 cells (ATCC number: HTB-54), which were mycoplasma-free, were maintained in culture at 37 °C, 5% CO_2 and 95% O_2 in McCoy's 5a medium with 10% fetal bovine serum, supplemented with 100 U/ml penicillin G and 100 μg/ml streptomycin sulfate. A549 cells (ATCC number: CCL-185) were maintained in culture at 37 °C, 5% CO_2 and 95% O_2 in Dulbecco's modified eagle medium (DMEM/F-12) with 10% fetal calf serum, supplemented with 50 U/ml penicillin G and 50 μg/ml streptomycin sulfate. Both cell types, maintained in T-75 flasks were passaged every 6 days. All the VEGF secretion and cytotoxicity studies were performed with cells grown in 96-well tissue culture plates. VEGF mRNA studies were done with cells grown in T-75 culture flasks.

2.3. RT-PCR for glucocorticoid receptor mRNA expression

To determine whether a signal for glucocorticoid receptor exists in Calu-1 and A549 cells, glucocorticoid receptor mRNA expression in these cells was determined using RT-PCR. Following total RNA isolation using RNA STAT-60™ RNA isolation kit (TEL-TEST, Friendswood, TX), RT-PCR was performed with an Access RT-PCR System (Promega, Madison, WI). RT-PCR for glucocorticoid receptor and GAPDH, an internal control, was performed in a standard 50 μl reaction mixture containing 0.2 mM deoxytriphosphate nucleotides, 2 mM $MgCl_2$, 20 pmol each of sense and antisense primers, 5 U of Tfl DNA polymerase, and 5 U of AMV reverse transcriptase. Amplification of the cDNA was performed as described previously by Bourcier et al. (1999). The amplified products were separated on a 2% agarose gel and visualized by staining with ethidium bromide. The sense and antisense primer sequences used for glucocorticoid receptor mRNA expression were ATGAGACCAGATGTAAGCTC and AATGCCATAAGAAACATCCA, respectively. The sense and antisense primer sequences used for GAPDH were CGATGCTGGCGCTGAGTAC and CGTTCAGCTCAGGGATGACC, respectively. Densitometric analysis for glucocorticoid receptor and GAPDH mRNA expressions was performed using Nucleovision™ Imaging System (Nucleotech, San Mateo, CA).

2.4. Effect of budesonide on VEGF secretion

On day 6, serum-containing medium was removed from Calu-1 and A549 cells plated in 96-well cluster plates and fresh medium without serum was added and the monolayers were allowed to remain in quiescence for 12 h. After the quiescence period, monolayers were incubated for 12 h with or without budesonide (10^{-13}–10^{-4} M) in serum-free medium. At the end of 12 h, VEGF secretion in the supernatants was quantified using an enzyme-linked immunosorbent assay (ELISA). Control cells were treated similarly in the absence of budesonide. Also, the role of glucocorticoid-receptor in budesonide effects was determined by performing VEGF secretion studies with 1 μM budesonide in the presence of 1 and 10 μM RU 486.

2.5. Effect of protein secretion modulators on VEGF secretion in Calu-1 and A549 cells

Following quiescence for 12 h in serum-free medium, both cell types were treated with golgi-disturbing agents, brefeldin A (10 μM) or monensin (10 μM), in serum-free medium for 12 h. Subsequently, secreted VEGF was quantified using an ELISA method as described below.

2.6. ELISA for VEGF quantification

Secreted VEGF in Calu-1 and A549 culture supernatants was quantified using an ELISA method. Coating antibody, detection antibody, and other necessary ELISA reagents were obtained from Research Diagnostics (Flanders, NJ). Briefly, supernatant samples of both cell types, standards, and blank samples were added to an ELISA plate, which was pre-coated overnight with the coating antibody (goat antihuman VEGF antibody), which recognizes $VEGF_{121}$ and $VEGF_{165}$ All the samples were allowed to incubate for 2 h with the coating antibody.

Subsequently, the plate was washed three times with wash buffer and the wells were incubated with the detection antibody (biotin-conjugated goat anti-human VEGF) for 2 h. Following secondary antibody incubation, the plate was incubated with poly-HRP80-streptavidin for 20 min. Subsequently, the plate was washed three times and allowed to incubate in the presence of substrate solution 3,3′,5,5′-tetramethylbenzidine (TMB-S) for 20 min until the reaction was stopped with sulfuric acid (0.5 M). All the absorbances were measured using a microtiter plate reader (Fischer Scientific, PA) with a test wavelength of 450 nm and a reference wavelength set at 550 nm.

2.7. Effect of budesonide and RU 486 on VEGF mRNA expression

Effect of budesonide with or without RU 486 co-treatment on VEGF mRNA expression was determined in Calu-1 and A549 cells. In brief, after an initial quiescence period of 12 h, both cell types were incubated with budesonide (1 μM) alone or with budesonide (1 μM) and RU 486 (10 μM) in serum-free medium for 12 h. At the end of this incubation, total RNA was extracted and RT-PCR for VEGF and GAPDH mRNA was performed.

2.8. Time-course of budesonide effect on VEGF mRNA expression and effect of cycloheximide co-treatment

Time-course of the effect of budesonide (1 μM) on VEGF mRNA expression was determined in Calu-1 and A549 cells. Following an initial quiescence period for 12 h, cells were incubated with budesonide (1 μM) in serum-free medium for 4, 8, and 12 h, respectively. At the end of these incubation periods, total RNA was extracted and RT-PCR was performed for VEGF mRNA expression as described below. Also, the effect of cycloheximide (10 μg/ml) co-treatment on budesonide-mediated VEGF and GAPDH mRNA expression was determined at the end of 12 h.

2.9. RT-PCR for VEGF mRNA expression

Following budesonide treatments, total RNA was isolated using the RNA STAT-60™ RNA isolation kit and RT-PCR was performed with an Access RT-PCR System. RT-PCR for VEGF and GAPDH, an internal control was performed in a standard 50 μl reaction mixture containing 0.2 mM deoxytriphosphate nucleotides, 2 mM $MgCl_2$, 20 pmol each of sense and antisense primer, 5 U of Tfl DNA polymerase, and 5 U of AMV reverse transcriptase. Amplification of the cDNAs was performed using specific VEGF and GAPDH primers as previously described (Boldrini et al., 1999). The amplification products were separated on 2% agarose gel and PCR products at 584, 510, 452, and 421 bp encoding $VEGF_{165}$, $VEGF_{145}$, $VEGF_{121}$, and GAPDH, respectively, and were visualized by staining with ethidium bromide. The sense and antisense primer sequences used for VEGF were TGGATCCATGAACTTTCTGCTGTC and TCACCGCCTTGGCTTGTCACAT, respectively.

2.10. Cytotoxicity

Effect of budesonide (0.01–100 μM) treatment on cytotoxicity was assessed by performing a colorimetric MTT assay. In this experiment, day 6 cells plated at a density of 1×10^4 cells/well in 96-well microtiter plates were used. At the end of the incubation period (12 h), 100 μl of MTT solution (5 mg/ml in serum-free medium) was added to each well and incubated for 4 h at 37 °C. After aspirating the supernatant, 100 μl of dimethyl sulfoxide (DMSO) was added to each well to dissolve the dark blue formazan crystals. The plates were shaken for 1 h to totally dissolve all the crystals. The absorbance was measured using a test wavelength of 450 nm and a reference wavelength of 650 nm using a microtiter plate reader (Fischer Scientific). The percentage of viable cells with all tested budesonide concentrations was calculated relative to untreated cells.

2.11. Statistical analysis

All data are expressed as mean ± S.D. or mean ± S.E.M. and comparison of the mean values was performed using a paired Student's *t*-test. Statistical significance was accepted at $P < 0.05$ level.

3. Results

3.1. Mechanisms of VEGF secretion from Calu-1 and A549 cells

To determine the mechanisms of VEGF secretion from Calu-1 and A549 cells, we examined the effects of brefeldin A and monensin on VEGF secretion (Fig. 1). Compared to

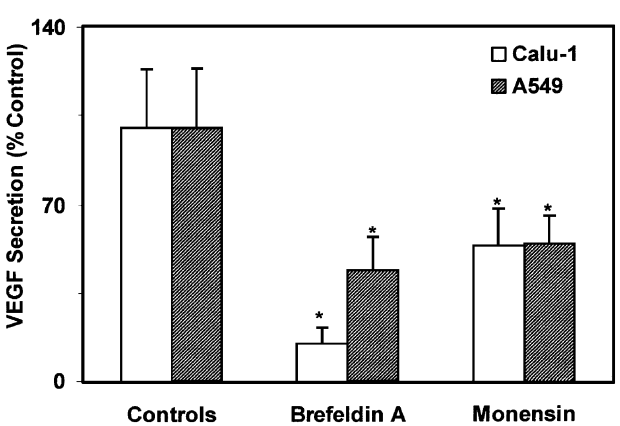

Fig. 1. Effect of protein secretion modulators on VEGF secretion from Calu-1 and A549 cells. Twelve hour VEGF secretion from Calu-1 and A549 cells was determined in the presence of 10 μM brefeldin A and 10 μM monensin. Data are expressed as mean ± S.D. for $n = 3$. "*" indicates significant difference from controls in both cell types.

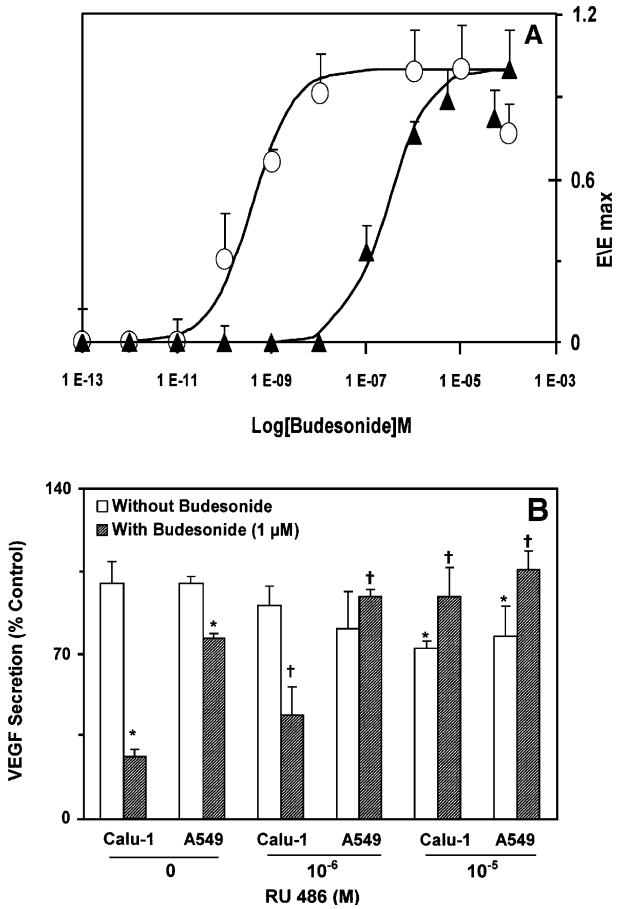

Fig. 2. Effect of budesonide and RU 486 on VEGF secretion from Calu-1 and A549 cells. (A) Concentration-dependent effect of budesonide on VEGF secretion from Calu-1 cells and A549 cells at the end of 12 h. Data are expressed as mean ± S.E.M. for $n = 3$ or 4. "○" indicates Calu-1 cells and "▲" indicates A549 cells. (B) Effect of RU 486 on budesonide-mediated inhibition of VEGF secretion from Calu-1 and A549 cells at the end of 12 h. Data are expressed as mean ± S.D. for $n = 3$ or 4. "*" indicates significant difference compared to 0 μM RU 486 without budesonide. "†" indicates significant difference compared to 0 μM RU 486 with budesonide. VEGF secretion was normalized to control groups receiving neither budesonide nor RU 486 treatment.

controls, brefeldin A as well as monensin, reduced VEGF secretion by 50% or higher in both cell types.

3.2. Budesonide inhibits VEGF secretion via its glucocorticoid receptor activity

Following 12-h budesonide (10^{-13}–10^{-4} M) treatment, VEGF levels in culture supernatants were quantified. In addition, to determine the role of glucocorticoid receptor activity in the observed effects, the effect of budesonide on VEGF secretion was determined in the presence of mifepristone (RU 486), a glucocorticoid receptor antagonist.

3.2.1. VEGF secretion in Calu-1 cells

Budesonide reduced VEGF secretion in a concentration-dependent manner, with the effects being significant at and above 0.1 nM (Fig. 2(A)). The EC_{50}, the concentration to attain the half-maximal response, and the E_{max}, the maximum decline in VEGF levels, were 0.8 nM and 1505 pg/mg protein, respectively. Co-treatment with 1 and 10 μM RU 486 significantly reversed the decline in VEGF secretion induced by 1 μM budesonide (Fig. 2(B)).

3.2.2. A549 cells

Budesonide reduced VEGF secretion in a dose-dependent manner, with the effect being significant at concentrations above 1 μM. The EC_{50} (Fig. 2(A)) and E_{max} values were 0.9 μM and 1527 pg/mg protein, respectively. Co-treatment with 1 and 10 μM RU 486 reversed the decline in VEGF levels induced by 1 μM budesonide (Fig. 2(B)). In both cell types, while 1 μM RU 486 treatment alone did not significantly inhibit VEGF secretion, 10 μM treatment alone significantly reduced VEGF secretion by 15–20% (Fig. 2(B)). Budesonide at 1 μM, the lowest concentration at which significant effects were observed in both cell types, was used in subsequent studies.

3.3. Glucocorticoid receptor mRNA expression in Calu-1 and A549 cells

RT-PCR analysis for glucocorticoid receptor mRNA expression indicated a single band at 598 bp (Fig. 3(A)), corresponding to the glucocorticoid receptor mRNA in Calu-1 (lane 2) and A549 (lane 4) cells. Densitometric

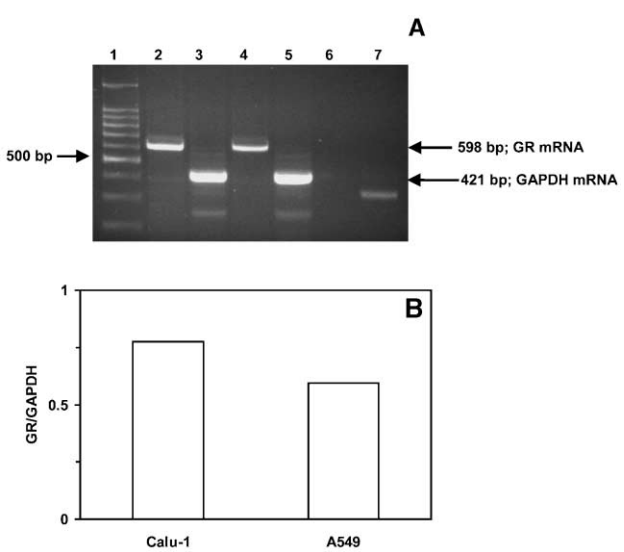

Fig. 3. Glucocorticoid receptor mRNA expression in day 6 Calu-1 and A549 cells. (A) RT-PCR for glucocorticoid receptor. The lane assignments are: lane 1, markers; lane 2, glucocorticoid receptor mRNA in Calu-1 cells; lane 3, GAPDH mRNA in Calu-1 cells; lane 4, glucocorticoid receptor mRNA in A549 cells; lane 5, GAPDH mRNA in A549 cells; lane 6, negative control (no RNA); and lane 7, kit control (330 bp). (B) Densitometric analysis of glucocorticoid receptor and GAPDH mRNA expression in Calu-1 and A549 cells. Data are expressed as the mean ratio of glucocorticoid receptor/GAPDH mRNA band intensity.

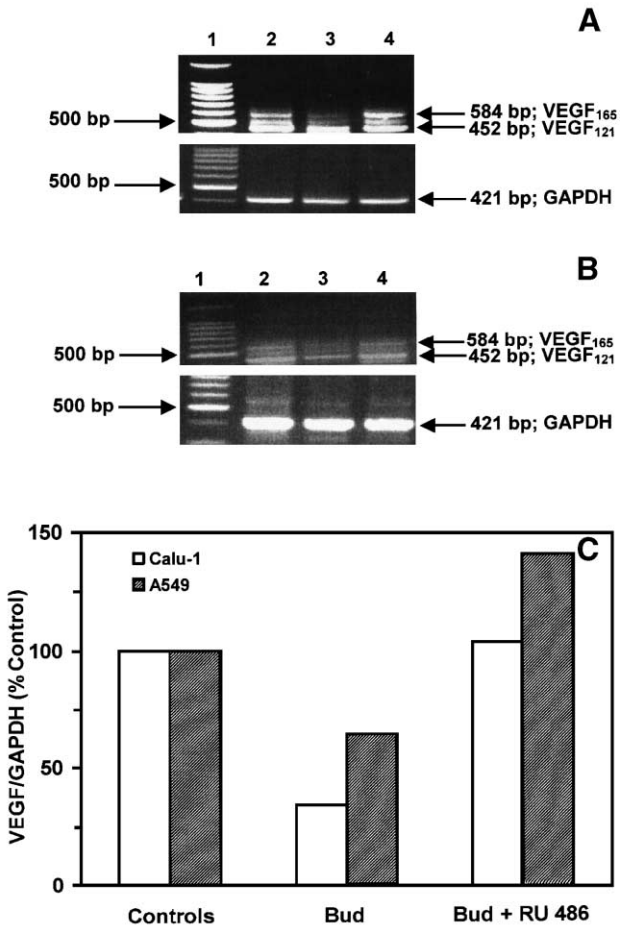

Fig. 4. Effect of budesonide with and without RU 486 on VEGF mRNA expression in Calu-1 and A549 cells. VEGF and GAPDH mRNA expression in (A) Calu-1 cells and (B) A549 cells. The lane assignments for VEGF and GAPDH expression in Calu-1 and A549 cells are: lane 1, molecular weight markers (100 bp); lane 2, controls; lane 3, budesonide (1 μM) treatment; lane 4, budesonide (1 μM) and RU 486 (10 μM) co-treatment. (C) Densitometric analysis of VEGF and GAPDH mRNA expression in Calu-1 and A549 cells. Data are expressed as the mean ratio of VEGF/GAPDH mRNA band intensity.

analysis (Fig. 3(B)) indicated a 20% higher glucocorticoid receptor mRNA expression in Calu-1 cells compared to A549 cells.

3.4. Budesonide inhibits VEGF mRNA expression via its glucocorticoid receptor activity

VEGF and GAPDH mRNA expression in Calu-1 and A549 cells indicated bands at 584, 510, 452, and 421 bp, corresponding to $VEGF_{165}$, $VEGF_{145}$, $VEGF_{121}$, and GAPDH, respectively (Fig. 4). Budesonide (1 μM) alone reduced VEGF mRNA levels and co-treatment with 10 μM RU 486 completely prevented this effect, suggesting the involvement of glucocorticoid receptor in budesonide-mediated effects. On the other hand, GAPDH mRNA expression was not affected by these treatments.

3.5. Budesonide inhibits VEGF mRNA expression in a time- and protein synthesis-dependent manner

Following 4-, 8-, and 12-h treatments with budesonide, a decrease in VEGF mRNA levels was observed in both cell types, with the decrease being greater in Calu-1 cells. In Calu-1 cells, the effect was observed at the end of 4 h of treatment, which persisted at 8 and 12 h (Fig. 5(A)). On the other hand, in A549 cells, the effect was pronounced only at the end of 8 h of treatment, and this effect persisted at 12 h (Fig. 5(B)). Interestingly, in both cell types, cycloheximide (10 μg/ml) co-treatment with budesonide

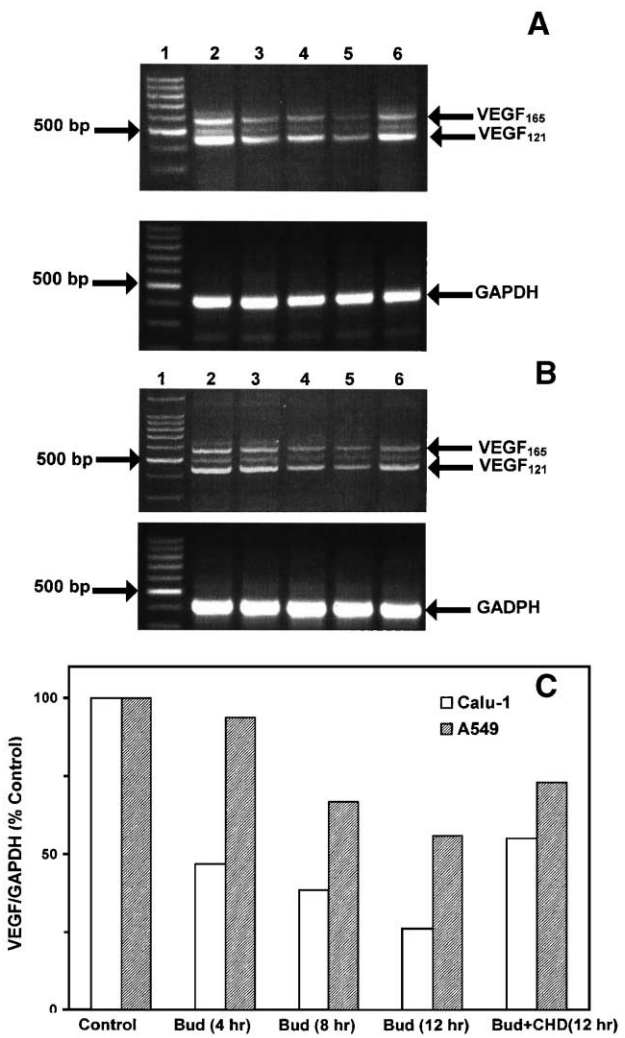

Fig. 5. Time-dependent effect of budesonide (1 μM) with and without cycloheximide on VEGF mRNA expression in (A) Calu-1 cells and (B) A549 cells. The lane assignments for VEGF and GAPDH expression in Calu-1 and A549 cells are: lane 1, molecular weight markers (100 bp); lane 2, controls; lanes 3, 4, and 5, budesonide (1 μM) treatment for 4, 8, and 12 h, respectively; and lane 6, budesonide (1 μM) and cycloheximide (10 μg/ml) co-treatment for 12 h. (C) Densitometric analysis of VEGF and GAPDH mRNA expression in Calu-1 and A549 cells. Data are expressed as the mean ratio of VEGF/GAPDH mRNA band intensity.

Table 1
Viability of Calu-1 and A549 cells after 12 h budesonide treatments as determined by MTT assay

Treatment (μM)	Calu-1[a]	A549 cells[a]
Controls	100 ± 5.11	100 ± 28
Budesonide (0.01)	92 ± 11	117 ± 25
Budesonide (0.1)	99 ± 10	109 ± 7
Budesonide (1)	104 ± 10	113 ± 26
Budesonide (10)	94 ± 10	83 ± 14
Budesonide (100)	94 ± 9	43 ± 5*

[a] The percentage of control absorbance values at 450/650 nm. All the absorbances were measured at a test wavelength of 450 nm and a reference wavelength of 650 nm. Data are expressed as mean ± S.D. for $n = 4$.

* Indicates significant difference from controls.

(1 μM) for 12 h reversed the VEGF mRNA expression, suggesting that budesonide-mediated effects require new protein synthesis.

3.6. Cytotoxicity

Cytotoxicity of budesonide in both cell types was determined as the relative formation of formazan following budesonide treatments when compared with controls treated with plain serum-free medium. In Calu-1 cells, budesonide treatment up to 100 μM did not show any significant cytotoxicity at the end of 12 h (Table 1). On the other hand, budesonide induced cytotoxicity in A549 cells at 100 μM.

4. Discussion

An important pharmacological effect of budesonide is its ability to reduce inflammation by inhibiting inflammatory mediators in epithelial and inflammatory cells (Barnes, 1998; Szefler, 1999). Budesonide inhibits edema and capillary hyperpermeability by inhibiting the release of inflammatory mediators such as tumor necrosis factor-alpha, interleukin-6, and interleukin-8 (Ek et al., 1999; Whelan et al., 1999). However, whether budesonide inhibits VEGF, a potent endothelial cell-specific mitogen capable of inducing vascular hyperpermeability and edema, is not known. Also, there are no reports on the effect of any corticosteroid on VEGF secretion or mRNA expression in pure respiratory epithelial cell preparations. Epithelial cells of the respiratory tract act as the first line of defense in inflammatory respiratory disorders and recruit inflammatory cells to the site of inflammation by releasing chemoattractants. Indeed, VEGF is a potent chemoattractant for monocytes (Clauss et al., 1990). Thus, the objective of this study was to determine the mechanisms of VEGF secretion in respiratory epithelial cells and to determine whether budesonide reduces VEGF secretion and expression in these cells.

To elucidate the mechanisms of VEGF secretion in respiratory epithelial cells, we determined VEGF secretion in the presence of monensin and brefeldin A. Monensin neutralizes the acidic intracellular vesicles and disrupts post-golgi endosomal structures and golgi sub-compartments and therefore, determines the role of these components in protein secretion (Dinter and Berger, 1998). On the other hand, brefeldin A inhibits protein transport between the endoplasmic reticulum (ER) and the golgi (Strous et al., 1993). In this study, brefeldin A, as well as monensin, significantly inhibited VEGF secretion from both cell types (Fig. 1), suggesting a role for intravesicular pH and ER–golgi pathway in VEGF secretion. Brefeldin A and monensin were previously shown to inhibit the secretion of signal sequence containing proteins such as interleukin-6, but not that of proteins such as interleukin-1 beta that lack signal sequences (Rubartelli et al., 1990; Sato et al., 1993). Signal sequence containing proteins are known to traverse the ER–golgi pathway. Thus, VEGF, which contains a putative N-terminal 26 amino acid hydrophobic secretory signal sequence (Cheung and Brace, 1998) is likely to traverse the ER–golgi pathway.

Budesonide inhibited VEGF secretion in a dose-dependent manner, with an EC_{50} of 0.8 nM and 0.9 μM in Calu-1 and A549 cells, respectively (Fig. 2(A)). Budesonide inhibited VEGF secretion via its glucocorticoid receptor activity. This is supported by the observation that inhibition of VEGF secretion by budesonide was reversed by co-treatment with RU 486, a glucocorticoid receptor antagonist (Fig. 2(B)). These results are consistent with Gloddek et al. (1999) and Heiss et al. (1996), who observed that RU 486 reversed dexamethasone-mediated reduction of VEGF levels in vitro and in vivo, respectively. RU 486 alone inhibited VEGF secretion at 10 μM (Fig. 2(B)), probably because of its partial glucocorticoid receptor agonist activity (Adcock et al., 1999; Schaison, 1989).

To investigate whether a decrease in VEGF mRNA expression is a mechanism by which budesonide inhibits VEGF secretion, VEGF mRNA expression in the presence of budesonide with or without RU 486 was determined. Budesonide decreased VEGF mRNA expression in both cell types and this inhibition was reversed by RU 486 co-treatment (Fig. 4). These observations are consistent with the effect of other corticosteroids such as dexamethasone, hydrocortisone, and cortisone, which reduced VEGF mRNA expression in human vascular smooth muscle cells (Nauck et al., 1997, 1998). Also, Heiss et al. (1996) reported that RU 486 reverses dexamethasone-mediated inhibition of VEGF mRNA expression in 9L cells. In both cell types, our observed VEGF mRNA inhibition (45–60%) at 12 h was comparable to the protein secretion inhibition (50–55%). Cycloheximide reversed budesonide-mediated inhibition of VEGF mRNA expression in both cell types (Fig. 5(A) and (B)), suggesting that budesonide-mediated VEGF mRNA inhibition in both cell types is dependent on protein synthesis, possibly the synthesis of appropriate transcription factors.

Inhibition of VEGF mRNA by budesonide most likely results from the binding of the budesonide–glucocorticoid

receptor complex to the AP-1 transactivator complex. As a result of this binding, the AP-1 transactivator complex is prevented from stimulating transcription of genes containing an AP-1 binding site (Jonat et al., 1990). Indeed, an AP-1 binding site is required for VEGF expression (Finkenzeller et al., 1995). Therefore, we speculate that budesonide is likely to inhibit the AP-1-dependent transcription of the VEGF gene in Calu-1 and A549 cells. As budesonide can inhibit cell growth (Namkung-Matthai et al., 1998), we investigated whether budesonide-mediated VEGF inhibition in Calu-1 and A549 cells is occurring as a result of decreased cell viability. Our MTT assay indicated that budesonide did not significantly decrease cell viability under the conditions of this study (Table 1).

Calu-1 cells were more sensitive to budesonide compared to A549 cells (Fig. 2(A)). Evidence in the literature suggests that the cellular sensitivity to glucocorticoids is directly proportional to the glucocorticoid receptor concentration (Oakley and Cidlowski, 1993). Our results indicated higher glucocorticoid receptor mRNA expression in Calu-1 cells (Fig. 3(A) and (B)), compared to A549 cells, consistent with the higher sensitivity of Calu-1 cells to budesonide. The greater sensitivity of Calu-1 cells can also be explained based on the observation that budesonide-mediated VEGF mRNA inhibition occurred early and to a greater extent in Calu-1 cells, when compared to A549 cells (Fig. 5(A) and (B)). In rat glioma cells and brain microvascular endothelial cells, dexamethasone inhibited VEGF secretion at and above 100 nM and 100 μM, respectively (Machein et al., 1999; Fischer et al., 2000). In this study, budesonide inhibited VEGF secretion at and above 0.1 nM and 1 μM in Calu-1 and A549 cells, respectively. This greater potency of budesonide can in part be due to the fact that budesonide exhibits higher glucocorticoid receptor affinity and tissue retention (Esmailpour et al., 1998; Miller-Larsson et al., 1998).

In conclusion, our results suggest that VEGF traverses the ER–golgi pathway during secretion from Calu-1 and A549 cells. In addition, budesonide inhibits VEGF secretion and VEGF mRNA expression in Calu-1 and A549 cells through its glucocorticoid receptor-mediated activity. Thus, budesonide may be of value in treating disorders of the respiratory tract such as pulmonary hypertension, wherein lung VEGF levels are elevated. As inflammatory disorders of the respiratory tract are characterized by vascular hyperpermeability and edema, and because VEGF is recognized as a principal contributing factor in these symptoms, it remains to be investigated whether the observed effects of budesonide contribute to the therapy of respiratory inflammatory disorders.

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