Late-breaking abstract: Differential expression profiles of genes involved in oxidative stress and inflammation in blood and sputum from healthy subjects and COPD patients

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Background: Environmental (mainly cigarette smoke) and genetic factors are known to be involved in the development of chronic obstructive pulmonary disease (COPD); however, a better understanding of the COPD genes expression dysregulation remains a major challenge. Increased oxidative stress is thought to be central in COPD pathogenesis and directly involved in local and systemic inflammation.

Methods: We have investigated, by RT-PCR array, the mRNA expression profile of 95 genes involved both in inflammation and oxidative stress in sputum and blood from COPD patients (n = 18) and healthy controls (n = 17). We have used Ingenuity Pathway Analysis Software (IPA) to identify the networks of interactions, the biological processes and pathways in which genes showing a significant expression modification are involved.

Results: In the blood cells of COPD, around half of genes showed modifications (26 up- and 19 down-regulated) compared to healthy controls and these were essentially involved in inflammation. Using IPA, we found that the most important cellular function altered was the cellular movement. In sputum cells, only 13 genes showed modifications (6 up- and 7 down-regulated, five were common with blood), most of them being involved in free radical scavenging and cell death.

Conclusions: Compared to healthy subjects, there was a clear dysregulation in gene expression at systemic level, and to a lesser extent, at airway level. Therefore, gene expression profile shows differences between local and systemic compartments.

Late-breaking abstract: Cyclooxygenase- and lypoxigenase-dependent generation of omega-3 electrophilic fatty acid-derivatives with anti-inflammatory properties

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Chronic obstructive pulmonary disease (COPD) is characterized by persistent inflammation of the airways and extensive oxidative damage. Activated macrophages and neutrophils are elevated in the airways of COPD patients where they sustain the inflammatory response and contribute to tissue damage. During inflammatory reactions arachidonic acid (AA) is released from cell membranes and is converted into the pro-inflammatory prostaglandins and leukotrienes by the action of
cyclooxygenase-2 (COX-2) and lypoxygenases (LO). It has been demonstrated that COX-2 and LO are active in many cellular processes, and their inhibition can contribute to limit tissue damage and inflammatory processes.

Data presented herein strongly suggest that electrophilic derivatives of omega-3 acid oxo-derivatives by the action of COX-2 and LO in activated human macrophages and stimulated neutrophils. These compounds displayed cytoprotective components deposition; activity of mitochondrial citrate synthase (CS), index of energy metabolism needs are satisfied in interstitial tissue structure (Rivolta, I et al. ERJ 2011; 37:943-9). On these samples, where the metabolic needs are limited to upper lobe while it contrarily occurs in lower lobe. We conclude that variances in tissue structure organization of the two lobes can be accounted for a topographic difference in the level of KGF expression to the upper lobe. We conclude that variance in tissue structure organization of the two lobes can be accounted for a topographic difference in the level of KGF expression. With respect to the upper lobe, KGF expression is predominantly important to respiratory mediators. Since avian epithelial cells from patients with asthma displayed enhanced IL-8 production we are assessing whether there is an intrinsic disturbance in the mRNA decay pathways. This work is supported by the Netherlands Asthma Foundation (3.2.06.031).

**P766**

**Late-breaking abstract: Surfactant protein D (SP-D) as a biomarker for mortality in elderly Danish twins**

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Engineered nanoparticles are widely used by the industry, however, it is not clear whether they possess a risk on respiratory health. The objectives of our study were to investigate effects of Titanium dioxide (TiO₂) and multi walled carbon nanotubes (MWCNT) on bronchial epithelial cell (BEC) viability and death. BEAS-2B cells and primary BEC obtained from both smokers and patients with COPD were incubated with 0.300g/ml TiO₂, and MWCNT for 24-48hrs. Cell viability was assessed by MTT, and apoptosis was analyzed by flow cytometry using Annexin V-FITC and 7AAD dyes. TiO₂ significantly decreased the viability of BEAS-2B cells at 100 (optical density (OD)=0.65, p<0.001) and 300 g/ml (OD=0.45, p<0.0001) concentrations after 24hrs as compared control cells (OD=0.85). Similarly, 100 and 300g/ml MWCNT decreased viability of these cells following 24 and 48hrs incubation. Although 300g/ml TiO₂ reduced the viability of primary BEC of smokers (OD=2.15 vs OD=2.5, p<0.0001), 300g/ml suppressed cell viability (OD=1.02; p<0.0001) after 24hrs. TiO₂ did not change the viability of BEC of COPD patients after 24hrs, whereas 300g/ml decreased viability of these cells (OD=0.40 vs 1.72; p<0.001) following 48hrs. Flow cytometry studies of BEAS-2B cells demonstrated that TiO₂ (300g/ml) decreases percentage of viable cells (90.66% vs 94.01%; p=0.0009), while inducing the percentage of late apoptotic (0.72% vs 4.22%; p=0.017) and necrotic (0.63% vs 4.03%; p=0.0099) cells. MWCNT also showed similar effects on apoptosis of BEAS-2B cells. These findings suggest that engineered nanoparticles may possess a risk on respiratory health by modifying viability and apoptosis of bronchial epithelial cells.

**P765**

**Late-breaking abstract: Lung regional differences in tissue adaptation to chronic hypoxia (CH)**

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Hypoxia (H) impacts to all body tissues which have to adapt to a decrease in O₂ delivery. In CH (10% O₂ for 3 weeks) two lung regions from rats, the upper and the lower lobe, showed differences in interstitial tissue structure (Rivolta, I et al. ERJ 2011; 37:943-9). On these samples, where the metabolic needs are satisfied in interstitial tissue structure (Rivolta, I et al. ERJ 2011; 37:943-9). On these samples, where the metabolic needs are satisfied in the upper lobe while it contrarily occurs in the lower lobe. We conclude that variance in tissue structure organization of the two lobes can be accounted for a topographic difference in the level of KGF expression. With respect to the upper lobe, KGF expression is predominantly important to respiratory mediators. Since avian epithelial cells from patients with asthma displayed enhanced IL-8 production we are assessing whether there is an intrinsic disturbance in the mRNA decay pathways. This work is supported by the Netherlands Asthma Foundation (3.2.06.031).

**P764**

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Engineered nanoparticles are widely used by the industry, however, it is not clear whether they possess a risk on respiratory health. The objectives of our study were to investigate effects of Titanium dioxide (TiO₂) and multi walled carbon nanotubes (MWCNT) on bronchial epithelial cell (BEC) viability and death. BEAS-2B cells and primary BEC obtained from both smokers and patients with COPD were incubated with 0.300g/ml TiO₂, and MWCNT for 24-48hrs. Cell viability was assessed by MTT, and apoptosis was analyzed by flow cytometry using Annexin V-FITC and 7AAD dyes. TiO₂ significantly decreased the viability of BEAS-2B cells at 100 (optical density (OD)=0.65, p<0.001) and 300 g/ml (OD=0.45, p<0.0001) concentrations after 24hrs as compared control cells (OD=0.85). Similarly, 100 and 300g/ml MWCNT decreased viability of these cells following 24 and 48hrs incubation. Although 300g/ml TiO₂ reduced the viability of primary BEC of smokers (OD=2.15 vs OD=2.5, p<0.0001), 300g/ml suppressed cell viability (OD=1.02; p<0.0001) after 24hrs. TiO₂ did not change the viability of BEC of COPD patients after 24hrs, whereas 300g/ml decreased viability of these cells (OD=0.40 vs 1.72; p<0.001) following 48hrs. Flow cytometry studies of BEAS-2B cells demonstrated that TiO₂ (300g/ml) decreases percentage of viable cells (90.66% vs 94.01%; p=0.0009), while inducing the percentage of late apoptotic (0.72% vs 4.22%; p=0.017) and necrotic (0.63% vs 4.03%; p=0.0099) cells. MWCNT also showed similar effects on apoptosis of BEAS-2B cells. These findings suggest that engineered nanoparticles may possess a risk on respiratory health by modifying viability and apoptosis of bronchial epithelial cells.
the spliced XBPI mRNA to validate the results. The spliced XBPI PCR product was also confirmed by DNA sequencing. The correlation of XBPI splicing with the induction of CHOP and BiP was r=0.962 (p<0.000) and r=0.884 (p<0.000), respectively. We compared the new method with the visualization of the spliced XBPI mRNA by gel electrophoresis and we obtained similar results. In conclusion, we have developed a simple and quantitative method for the detection of spliced XBPI mRNA.

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P769 Pulmonary apoptosis in fetal Down syndrome
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Background: Increased levels of apoptosis have been implied in various non-pulmonary conditions frequently found in Down syndrome (DS). Children with DS are at increased risk for acute lung injury and fetal lung development is disrupted processes, apoptosis plays a key role. Nevertheless, pulmonary apoptosis has not been studied in DS.

Aim: We hypothesized that the amount of apoptotic epithelial cells in fetal lungs of DS is increased compared to controls.

Methods: We compared lung tissue sections from autopsies of 21 fetuses with DS and 12 controls (16-24 weeks gestational age (GA)). Sections were double stained with antibodies against pan-cytokeratin (CK) and activated caspase-3 (C3), markers for epithelium and apoptosis. Per section, 7 random photographs were taken at 200x magnification. Spectral imaging software was used to quantify the mean number of pixels that showed colocalization of CK and C3. All sections were H&E stained to determine the presence of canalicular or saccular morphology.

Results: The mean (SD) percentage of CK-positive pixels was equal between DS and controls (27.2% (4.7) versus 27.1% (6.2); p=0.97). The median percentage (IQR) of CK-positive pixels that showed colocalization of C3 was 0.16% (0.18) in DS compared to 0.27% (0.24) in controls (p=0.45). This was independent of gestational age.

Conclusion: The number of apoptotic epithelial cells in lungs of DS fetuses does not differ from controls. We did not find a difference in the development of epithelial structures in DS compared to controls. This might explain anomalies in alveolar development found at birth in DS.

P770 Airway epithelial protocadherin-1 expression is regulated by house dust mite and cigarette smoke exposure in mice
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Introduction: Airway epithelial protocadherin-1 (PCDH1) is expressed in airway epithelial cells and encodes two isoforms of a protocadherin transmembrane protein. We aim to get insight into PCDH1 function in airway epithelial cells in relation to BHR. Therefore, we analyzed in vivo regulation of Pcdh1 isoforms in lungs under basal conditions and in mouse models of short-term cigarette smoke exposure and asthma in mouse models of short-term cigarette smoke exposure and asthma.

We identified a novel isoform of Pcdh1 lacking the transmembrane domain but re-localized in the intracellular signalling motifs, indicating a novel function as signalling adapter molecule. Bronchial epithelial cells expressed all isoforms of Pcdh1, while airway smooth muscle only expressed the isoforms encoding the signal transduction domains. Interestingly, Pcdh1 expression was unaltered during oxidative stress, but increased after termination of the treatment, indicating a putative role in epithelial repair. In strong contrast, Pcdh1 mRNA expression was markedly reduced by CS exposure, as soon as 6 hours after a single exposure. These latter data are especially of interest given the initial identification of linkage to the PCDH1 region in CS-exposed families. We conclude that CS-induced changes in airway epithelium directly affect Pcdh1 expression levels, and hypothesize that PCDH1 regulation contributes to the epithelial response to CS-induced injury.

P771 Fluticasone furoate restores leptin/leptin receptor pathway in nasal epithelial cells
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Leptin/leptin receptor pathway has been shown to be involved in the epithelial homeostasis and in tissue repair. Allergic rhinitis (AR) is characterized by the late, immediate inflammation induced by the allergen exposure, leading to a chronic inflammation with consequential structural abnormalities in the nasal epithelium. Topical corticosteroids are recommended as first-line therapy in AR. The role of the leptin/leptin receptor pathway and the specific effects of Fluticasone furoate (FF), a new topical corticosteroid, in the homeostasis of nasal epithelial cells are largely unknown. We aimed to determine whether a nasal epithelial dysfunction of leptin/leptin receptor pathway contributes to AR pathogenesis and to investigate the effect of FF on this pathway. The human nasal epithelial cell line RPMI 2650 was first examined for leptin/leptin receptor expression by immunocytochemistry and by flow-cytometry. Then, the RPMI 2650 cells were cultured in the presence or absence of the allergen extract fromAmbrosia artemisiifolia (PAR1), of the fibrogenic cytokine TGF-β1 and of FF and analyzed for leptin receptor by flow-cytometry and for cell proliferation by clonogenic assay. The RPMI 2650 cells express leptin receptor. PAR1 and TGF-β1 significantly decreases the leptin receptor expression and cell proliferation and FF completely abolishes and reverts the effects of both PAR1 and TGF-β1. In conclusion, allergen exposure and TGF-β1 alter the homeostasis of nasal epithelia by down-regulating leptin/leptin receptor pathway whereas FF is able to restore both this pathway and nasal epithelial homeostasis.

P772 S-CMC-Lys reduces oxidative stress of respiratory cells and increases GSH intracellular content
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The treatment of human respiratory cells with S-carboxyethyl lysine salt monohydrate (S-CMC-Lys) determines an increase in GSH secretion by stimulating glutathione (GSH) efflux from cells. The aim of this study was to evaluate the S-CMC-Lys effects on the GSH intracellular content and metabolism after an oxidative stress or after exposure to Cigarette Smoke Extract (CSE). Methods: GSH and Reactive Oxygen Species (ROS) content in response to S-CMC-Lys, H2O2 and CSE treatments was evaluated with fluorometric or luminescence-based assays in respiratory cell lines. The expression of GSH system enzymes was detected by real-time PCR and western immunoblotting.

Results: S-CMC-Lys induces in respiratory cells lines a significant increase of the GSH intracellular content and a significant increase in the expression of the catalytic, GRIAC Research Institute (γ-Glutamyl Cysteine Synthase), a key enzyme for the synthesis of GSH. S-CMC-Lys pre-treatment of cells prior H2O2 exposure was able to reduce ROS. The co-sublimination of S-CMC-Lys with CSE for a prolonged period (24 hours) resulted in a increase in the GSH content and a significantly increased level of γ-GCS and GR (glutathione reductase) mRNA. Conclusions: S-CMC-Lys increases the GSH intracellular content of respiratory cell lines by enhancing the expression of γ-GCS and catalytic subunits. The pre-treatment of respiratory cells with S-CMC-Lys upregulated a protective function during oxidative stress, reducing the ROS-mediated inflammatory response. The co-sublimination of S-CMC-Lys potentiates the cell adaptive response to CSE exposure, counteracting the CSE negative effects on the GSH system and ROS production.

P773 Mitochondrial dysfunction in airway epithelium increases pro-inflammatory IL-8, impairs barrier function and reduces glucocorticoid responsiveness
Roland Hoffmann, Simonne Brandenburg, Nick ten Hacken, Antoon van der Linde1, Irene Heijink.

Introduction: Despite the broad anti-inflammatory effects of glucocorticoids (GC), they provide little therapeutic benefit in COPD. Mitochondrial dysfunction has been described in COPD patients and can be induced in airway epithelial cells in vitro by cigarette smoke. We hypothesize that impaired mitochondrial function induces a phenotypic shift in airway epithelial cells leading to increased pro-inflammatory responses, impaired barrier function and reduced GC sensitivity. Methods: We determined IL-8 secretion (ELISA) and epithelial barrier function (ECIS) in the alveolar epithelial cell line A549 and the mitochondrial impaired cell line A549-B2 and their sensitivity to the GC budesonide (10−8M).
Effect of phosphodiesterase IV inhibitors on eotaxin expression in bronchial epithelial cells — Comparison between immortalized and primary line

Magdalena Paplinska, Ryszarda Chazan, Hanna Grubek-Jaworska.

Eotaxins are an important group of the patophysiology in the obstructive airway diseases. They are responsible for eosinophil recruitment into respiratory tract. Phosphodiesterases (PDEs) are a huge and diversified family of enzymes degrading cAMP. PDE4 inhibitors as drugs, act through cAMP elevation and can inhibit inflammation in many ways.

The aim of this work was to evaluate the effect of PDE4 inhibitors (rolipram and RO-20-1724) on eotaxin (CCL11, CCL24 and CCL26) expression in human bronchial epithelial cells immortalized - BEAS-2B (ATCC) and primary (ATCC). Cells were preincubated with PDE4 inhibitors for 1 h and stimulated with IL-1+TNF-α or IL-13+TNF-α for 48 h. Protein levels were measured using ELISA kits, changes in genes expression were measured using real time PCR.

Results: The both of cell lines produced different eotaxins: BEAS-2B synthesized CCL11 and CCL26. Distinct effects of PDE4 inhibitors in immortalized as compared to primary cell line were observed. PDE4 inhibitors decreased the mean level of eotaxin gene and protein expression in BEAS-2B usually in statistically significant manner. PDE4 inhibitors stimulated eotaxin gene expression; specially rolipram significantly increased the mean levels of eotaxin expression, but did not change their protein synthesis.

Conclusion: Our results do not permit to define the effect of PDE4 inhibitors on bronchial epithelial cells because of the differences in the biology of the both used cell lines. Unequivocal resolution of the problem needs more experimental trials using more primary cell lines or experimental animal model.

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Interaction between epithelial cells and neutrophils during pro-inflammatory conditions

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Inter-cellular communication is essential for defense and survival of the organism. The aim of the study was to find out whether there is an active cross-talk between cells constituting the first line of defense; alveolar epithelial cells (A549) and neutrophils, following activation with pro-inflammatory stimuli in vitro. Further, to explore whether this communication is altered in chronic obstructive pulmonary disease (COPD), a condition characterized by chronic airway and lung inflammation.

Endothelial neutrophils from healthy subjects and COPD-patients were co-cultured with A549 cells in medium and in medium containing lipopolysaccharide (LPS), peptidoglycan (PGN) or tumor necrosis factor (TNF). The expression of TLR2, TLR4 and CD14 on the cell surface of neutrophils was assessed by flow cytometry and CXCL8 (IL-8) and soluble CD14 (sCD14) in the supernatant were measured with ELISA.

On neutrophils, the surface expression of TLR2 was diminished following activation with all three pro-inflammatory stimuli and membrane bound (mCD14) and TLR4 expression increased in co-cultures compared to single cell cultures, irrespective of pro-inflammatory stimulation. A strong correlation between CXCL8 and sCD14 was observed in LPS-stimulated co-cultured A549 and neutrophils. These data showed a down regulation of TLR2 on neutrophils induced by pro-inflammatory stimuli and is strongly suggesting an active cross-talk between A549 cells and blood neutrophils, both in unstimulated and following activation with pro-inflammatory stimuli, in vitro.

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Effect of cigarette smoke extract or TGF-β1 on hyaluronan production and hyaluronan modulating enzymes in primary murine lung fibroblasts

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Hyaluronan (HA) is a component of the extracellular matrix and low molecular weight (LMW) HA fragments have pro-inflammatory capacities. Exposing mice to cigarette smoke (CS) for 1 or 6 months results in enhanced deposition of LMW HA in lung parenchyma and airway walls and in altered expression of HA syntheses and hyaluronidases (Bracke et al., Am J Respir Cell Mol Biol. 2010;42(6):753-61). To pinpoint a source of HA, we studied HA-production and hyaluronan-modulating enzymes in primary murine pulmonary fibroblasts stimulated with cigarette smoke extract (CSE) or TGF-β1.

Fibroblasts were isolated from lungs of C57BL/6j mice and cultured in vitro. At passage 6, cells were stimulated for 24 or 48 h with CSE or TGF-β1, 5% CSE or 2ng/ml TGF-β1. mRNA expression of HA syntheses (Has1, Has2, Has3) and hyaluronidases (Hyal1, Hyal2) was evaluated by RT-PCR. HA production was measured in supernatant by ELISA.

In vitro stimulation of pulmonary fibroblasts with CSE significantly decreased the mRNA expression of Has1 (synthesizing high molecular weight (HMW) HA) and significantly increased the expression of Hyal2 (degrading HMW HA to LMW HA fragments). Stimulation with TGF-β1 resulted in significantly increased mRNA expression of Has2 (synthesizing HMW HA). Accordingly, HA-levels in the fibroblast supernatant decreased significantly upon 48h stimulation with CSE, while they were significantly increased upon 24 or 48h stimulation with TGF-β1. Decreased Has1 and increased Hyal2 in CSE-stimulated fibroblasts suggests reduced synthesis and enhanced breakdown of HMW HA. This may contribute to the accumulation of LMW HA fragments, observed in CS-exposed mice.

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Cigarette smoke down-regulates the expression of β-catenin in primary human lung fibroblasts

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Rationale: Cigarette smoke is the major cause of COPD/emphysema but the etiology of these diseases is still unknown. β-catenin is a signaling molecule which is regulated through degradation/stabilization mechanism, and which promotes cell proliferation via the Wnt signaling pathway. Decreased β-catenin signaling may be involved in the parenchymal tissue death leading to emphysema.

Objectives: Investigate the effect of cigarette smoke on the expression of β-catenin and cell proliferation in primary human lung fibroblasts (n=6).

Methods: Fibroblasts were exposed to cigarette smoke conditioned medium (20%, 128s)
24 hours). Expression of β-catenin was determined by immuno-blotting. Proliferation was determined by [3H]-thymidine incorporation.

**Results:** Cigarette smoke significantly down-regulated β-catenin expression and reduced proliferation of primary lung fibroblasts.

**Conclusions:** Cigarette smoke down-regulates β-catenin and reduces cell proliferation in lung fibroblasts which may underlie the impaired tissue repair leading to parenchymal destruction in the lung of COPD/emphysema patients.

**P779**

Anti-inflammatory effect of beclomethasone dipropionate and formoterol on TNF-α-induced human endothelial cell activation

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**Background:** Among the structural cells, endothelial cells (EC) represent critical elements: they control leukocyte traffic through the adhesion molecule and chemokine expression.

**Aim:** To investigate the effect of beclomethasone dipropionate (BDP) and formoterol (F), either alone or in combination, on TNF-α-induced ICAM-1 expression and IL-8 release in human umbilical vein endothelial cells.

**Methods:** EC were incubated with BDP (10^{-11}-10^{-5} M), F (6/100 with respect to BDP concentration) or drug diluent (control cells: CC) and then exposed to TNF-α (100 U/ml; 4 hrs). For BDP/F combination (w/w: 100/6) EC were treated with low doses of BDP (10^{-12} and 10^{-10} M) and/or FOR (6×10^{-13} and 6×10^{-11} M). Surface ICAM-1 expression and IL-8 release were measured by ELISA.

**Results:** BDP reduced TNF-α-induced IL-8 release (mean±SEM% decrement) with maximal inhibition 23.7±4.2 at 10^{-7} M and 22.6±3.7 at 10^{-5} M (p<0.05 vs CC); F did not significantly affect IL-8 release (9.2±2.8% at the maximal dose tested). In a different experimental set, BDP/F inhibited IL-8 release with respect to BDP alone (10^{-7} M/6×10^{-11} M: 27.6±4.7 vs 10^{-7} M: 14.6±3.5%, p<0.05, n=8), achieving an effect comparable to that observed with BDP 10^{-5} M alone: BDP/F, although to a less extent, tended to decrease ICAM-1 expression (10^{-7} M/6×10^{-11} M: 17.2±0.8 vs 10^{-7} M: 9.3±2.1% and F:4.2±2.6, p=0.05, n=5).

**Conclusions:** BDP in combination with F is more effective in inhibiting EC activation as compared with BDP alone, thus allowing to use lower BDP doses to reach the maximum inhibitory effect. These results may explain some clinical anti-inflammatory activities of BDP/F combination.