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Suppression of interleukin (IL)-8 and human beta defensin-2 secretion in LPS-and/or IL-1β-stimulated airway epithelial A549 cells by a herbal formulation against respiratory infections (BNO 1030)

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ABSTRACT

Aim of the study: A special ethanolic-aqueous extract from seven traditional medicinal plants (BNO 1030) has been used for several decades to treat recurrent infections of the respiratory tract. Considering the potential role of interleukin-8 (IL-8) and human beta defensin-2 (hBD-2) in inflammation, we investigated the effect of BNO 1030 on lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* or IL-1β-induced inflammatory mediators in A549 human type II alveolar epithelial cells.
Materials and methods: A549 cells were stimulated with LPS (100 μg/ml) or IL-1β (50 ng/ml) in the presence of the preparation and the secretion of IL-8 and hBD-2 were measured after 18 h and 24 h in cell free supernatants using enzyme-linked immunosorbent assays (ELISA). Cell viability and cell growth was investigated by propidium iodide uptake and WST-1 assay, respectively.
Results: BNO 1030 inhibited the secretion of IL-8 and hBD-2 at non-cytotoxic concentrations (0.1–100 μg/ml; cell growth inhibitory concentration, 50% (IC_{50}) = 678 ± 87.6 μg/ml). Stimulation by IL-1β led to a 7-fold activation of IL-8 secretion, which was reduced by 37.7 ± 4.1% ($p < 0.05$) after incubation with 100 μg/ml BNO 1030. Inducible hBD-2 was suppressed by 91.8 ± 15.6% ($p < 0.01$) at the same concentration of BNO 1030 (IC_{50} = 0.7 ± 0.1 μg/ml). The 2-fold increase of IL-8 secretion by LPS-stimulated cells was completely abolished at concentration of 50 μg/ml BNO 1030 (IC_{50} = 5.7 ± 3.6 μg/ml).
Conclusion: BNO 1030 suppressed the secretion of IL-8 and hBD-2 in cultured epithelial A549 cells. These results support its use as a phytotherapeutic product prepared from traditional remedies in inflammatory diseases, especially those affecting the respiratory tract.

1. Introduction

The herbal medicinal product Imupret® (formerly Tonsilgon®) oral drops had been placed in the German market before the German Drug Law came into force. The composition presumably goes back to the nineteen-thirties, it has been at least available since 1948 with the tradename "Drüsin II" until it was renamed to "Tonsilgon" in 1965. The indication at those times reads as follows: recurrent and chronic respiratory infections, especially tonsillitis. Since this registration the product is continuously on the German market, by now more than 45 years, but actually the product is in use for a much longer time. Imupret® is a traditional herbal medicinal product which in particular proves not to be harmful in the specified conditions of use and the pharmacological effects or efficacy of the medicinal product are plausible on the basis of long-standing use and experience. BNO 1030 (proprietary herbal medicinal product: Imupret® or Tonsilgon® N oral drops) is a special ethanolic-aqueous extract of seven medicinal plants: marshmallow root (Althaeae radix), chamomile flower (Matricariae flos) yarrow herb (Millefolii herba), oak bark (Quercus cortex), walnut leaves (Juglandis folium), horsetail herb (Equiseti herba), and dandelion herb (Taraxaci herba). Already in the ancient writings of the Greeks and Romans (e.g. Dioscurides, Plinius) their use and benefits in folk medicine were described (Wichtl, 1984, 1989; Benedum et al., 2006). Modern review of phytochemical investigations, pharmacological properties and clinical particulars evidenced and justified the use of each single herbal component. For each of them, a broad spectrum of pharmacological effects including immunostimulatory, antiseptic, and anti-inflammatory properties could be documented (Hu and Kitts, 2003; Do Monte et al., 2004; Liu et al., 2010). Due to its observed effect on lymphatic system, alleviation of the irritated oral and pharyngeal mucosa as

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well as anti-inflammatory effect, BNO 1030 has been used for the treatment of acute and recurrent infections of the respiratory tract since the 1960s (Maier and Maier, 1974; Sprenger, 1975; Berger, 2008).

The respiratory epithelium is the first tissue to confront inhaled pathogens. This is a critical position for the innate immune system, representing the first line of host defense against invading microorganisms. Furthermore, in response to inflammatory stimuli, pulmonary epithelial cells also generate various immune effectors that include cytokines, chemokines, and antimicrobial peptides (Strieter et al., 2002). *Pseudomonas aeruginosa* (*P. aeruginosa*) is an important respiratory pathogen. Pulmonary epithelial A549 cells respond to *P. aeruginosa* via the Toll-like receptor (TLR)-4 and activation of nuclear factor kappa B (NF-κB), which upregulates several pro-inflammatory genes, including that coding for IL-8 (Pechkovsky et al., 2000).

Interleukin-8 (IL-8), also known as neutrophil-activating peptide 1, (NAP1), is a CXC chemokine that is recognized as a potent effector of neutrophil functions (Baggiolini et al., 1994). IL-8 specifically attracts several cell types involved in inflammation. After NF-kB activation by cytokines or lipopolysaccharide (LPS), IL-8 expression increases (Strieter et al., 2002) and this cytokine has been implicated as a causative agent in a broad range of pathological conditions including rhinitis, bronchitis, and bacterial infections (Benson et al., 1999; Hill et al., 1999).

Human β-defensin (hBD)-2, is an antimicrobial peptide primarily induced in epithelial cells that plays a unique role linking innate and acquired immunities (Schutte and McCray, 2002). Defensins are small, arginine-rich cationic peptides which express antimicrobial activity against gram-positive and -negative bacteria, fungi and certain viruses. In humans, defensins are divided into two subgroups, α- and β-defensins. The hBD-1 is constitutively expressed by epithelial cells and is not upregulated by proinflammatory stimuli or bacterial infection. In contrast, hBD-2 is highly inducible by inflammation and is involved in the resistance of epithelial surfaces to microbial colonization. Low basal hBD-2 mRNA expression has been detected in the skin, lung, and trachea epithelial cells, but was strongly upregulated in response to proinflammatory stimuli in the epithelium of the respiratory tract (Schutte and McCray, 2002). The TLR4 plays a critical role in LPS- induced hBD-2 expression by human A549 epithelial cells (MacRedmond et al., 2005, 2007).

Elevated levels of inducible hBD-2 have also been found in the plasma of patients with various inflammatory lung diseases (Hiratsuka et al., 1998; Dauletbaev et al., 2002).

In this study, we have investigated the effect of BNO 1030 on the secretion of IL-8 and hBD-2 release in a transformed alveolar epithelial cell line A549, which most resembles type II cells in the response to LPS and IL-1β. We selected to study BNO 1030 because of its ethnopharmacological background to the traditional use of single herbs in ancient Europe.

2. Materials and methods

2.1. Extract and agents

BNO 1030 is a special ethanolic-aqueous extract. 100 g of the herbal medicinal product Imupret® contain an extract of marshmallow root (0.4 g), chamomile flower (0.3 g), horsetail herb (0.5 g), walnut leaves (0.4 g), yarrow herb (0.4 g), oak bark (0.2 g), and dandelion herb (0.4 g). BNO 1030 extract [batch 660476] was obtained from Bionorica SE (Neumarkt, Germany) and dissolved in 70% (v/v) ethanol. The extract at a concentration of 10 mg/ml was heated for 5 min at 60 °C and sonicated for 45 min. Solubilised solution was lyophilized by low degree. Stock solution at concentration of 20 mg/ml (Hank's balanced salt solution) in aliquots was stored at −20 °C. LPS from *P. aeruginosa*, recombinant human IL-1β expressed in *Escherichia coli*, dexamethasone (DEX) and polymyxin B sulphate were purchased from Sigma (Buchs, Switzerland). Tetrazolium salt WST-1 from Roche Diagnostics (Rotkreuz, Switzerland) was used.

2.2. Epithelial cell culture

Type II-like human lung epithelial cells A 549 (ATCC CCL 185) were obtained from ATCC (Rockville, MD, USA). For routine maintenance, the A549 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine and penicillin/streptomycin 1% in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37 °C. At about 70–80% confluence, cells were detached with trypsin/EDTA solution to form a cell suspension and counted using a haemocytometer before inoculation. Cell viability was higher as 95% using trypan blue dye exclusion in all experiments. Cultured A549 cells expressed TLR4 as confirmed using the indirect immunofluorescent staining with the mouse anti-human TLR4 monoclonal antibody HTA125 (BD Biosciences Pharmingen, Basel, Switzerland) and flow cytometric analysis (data not shown). All cell culture reagents were from Sigma. In all experiments the amount of FCS was reduced to 2% in the assay medium. The final concentration of solvent in culture medium did not exceed 0.5% (v/v). This concentration did not affect the viability or cytokine production of the investigated cells.

2.3. Cell viability and growth assay

The effect of the BNO 1030 preparation at 1 and 2 mg/ml on cell viability (20×10^4 ml^{-1}) after 24 h was investigated by the live-dead discrimination with propidium iodide (PI; Sigma) at a final concentration of 5 μg/ml using flow cytometry as previously described (Hostanska et al., 2007). In order to determine the effect of compound on cell growth, treated cells at a density of 3000 cells/well were incubated in microtiter plates for 20 h followed by 4 h with WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate). The cleavage of WST-1 to formazan by metabolically active cells was quantified by scanning the plates at 450 nm and 650 nm reference wavelength in a microtiter plate reader. Test medium was used as background control. Three independent set of experiments performed in triplicate were evaluated. Controls with the solvents ethanol were tested in parallel. The growth inhibition rate was calculated as percentage of parallel untreated controls. The IC_{50} values were evaluated from the dose–response curves of each experiment using Microsoft-Excel computer software. The direct reductive potential of the test substances was tested concomitantly in a cell free system.

2.4. Detection of IL-8 cytokine and human β-defensin-2 production

IL-8 cytokine and hBD-2 secretion were measured in the supernatants of A549 cells (8×10^4) after 18 h and 24 h incubation, respectively, with different concentrations (0.1–100 μg/ml) of preparation alone and/or after stimulation with LPS (100 μg/ml) and IL-1β (50 ng/ml) performed in duplicate using Instant IL-8 ELISA (Bender Medsystems, Vienna, Austria) and human β-defensin 2 ELISA kit (Phoenix Pharmaceuticals Inc., Burlingame, USA), according to manufacturer's instructions. The sensitivity of the assay was 1.3 pg/ml for IL-8 and 7.8 pg/ml for hBD-2. Supernatants were collected and stored frozen at −80 °C until use. As positive control 10^{-7} M DEX was used. Protein concentrations in supernatants were determined by Lowry assay using Bio-Rad DC

protein Assay (Biorad, Hercules, USA). Bovine serum albumin was used as standard. To rule out the possible endotoxin contamination in the herbal product it was pre-treated with polymyxin B (5 μg/ml) in some experiments.

2.5. Statistics

Results were shown as mean ± standard deviation. One-way analysis of variances (ANOVA) and Student's two-tailed test was employed to assess the statistical significance of differences between the respective means and its untreated (WST-1 assay) or stimulated (detection of IL-8 and hBD-2) controls. IC_{50} values were obtained by linear or exponential regression analysis of data using the Microsoft Excel software. Probability values equal to or less than 0.05 were considered significant.

3. Results

3.1. Effect of BNO 1030 on the viability and cell growth of A549 cells

To exclude the possibility that cell toxicity might have been responsible for any decrease in IL-8 and hBD-2 levels, the viability of extract-treated A549 cells was evaluated using PI uptake. The membrane of cells was not affected up to concentrations of 2000 μg/ml of BNO 1030 when examined by inverted-phase contrast microscopy. Thus, we investigated PI uptake in A549 cells treated for 24 h with only two concentrations (1000 and 2000 μg/ml) of BNO 1030 by flow cytometry. No toxicity of BNO 1030 was observed up to concentration of 2000 μg/ml. The viability of cells at 2000 μg/ml BNO 1030 was 96.6 ± 2.3% normalized to the untreated control (92.8 ± 3.5%). Cell growth was measured indirectly by the cleavage of WST-1 to formazan by cellular enzymes of metabolically active cells after 24 h exposure of A549 cells to BNO 1030 (0.1–1000 μg/ml) in the presence and/or absence of LPS (100 μg/ml). Inhibition of cell proliferation by BNO 1030 was dose dependent. The IC_{50} concentrations of BNO 1030 was 678.3 ± 87.6 μg/ml in the absence and 796.2 ± 93.5 μg/ml in the presence of LPS (Fig. 1).

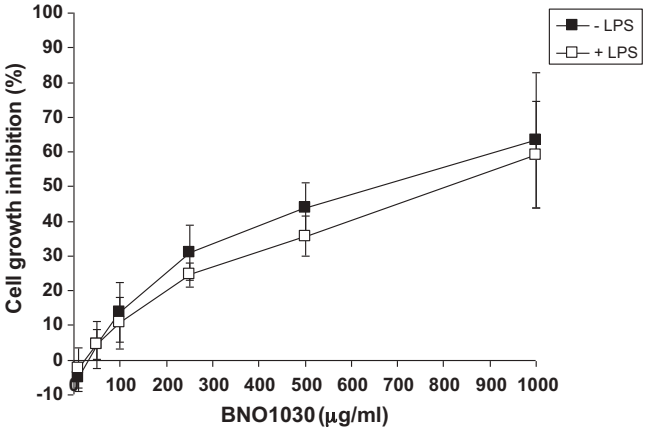

Fig. 1. Effect of BNO 1030 on cell growth of A549 cells. Respiratory A549 cells were treated for 24 h with indicated concentrations of BNO 1030 in the absence or presence of LPS (100 μg/ml) and the growth inhibition was determined by WST-1 assay. Untreated cells (in medium only) and cells incubated in the presence of vehicle (0.5%, v/v ethanol) were used as controls. Dose–response curves from triplicates repeated three times with similar results are expressed as mean growth inhibition percentage ± SD. The solvent 0.5% ethanol alone did not affect the growth of A549 cells (mean = 3.8 ± 2.1%). IC_{50} values derived for growth inhibition in A549 cells were 678.3 ± 87.6 μg/ml or 796.6 ± 93.4 μg/ml in the absence or presence of 100 μg/ml LPS.

3.2. Inhibitory effect of BNO 1030 on LPS and IL-1β-induced cytokine production in A549 cells

Exogenous stimuli like bacterial LPS possess the potential to activate human A549 epithelial cells and induce the secretion of IL-8, which can be used as a marker for A549 cell activation. To answer whether BNO 1030 can modulate the IL-8 production by A549 cells, we treated LPS (100 μg/ml) or IL-β (50 ng/ml)-activated cells with increasing concentrations of BNO 1030 (0.1–100 μg/ml) and measured the protein level of IL-8 in the supernatants by ELISA. Based on the LPS titration assay, 100 μg/ml LPS from *P. aeruginosa* was used throughout the experiments. To exclude the presence of contaminating endotoxin in the preparation, it was pre-incubated with polymyxin B (5 μg/ml) and then assayed for cytokine induction in IL-1 β-activated A549 cells. No difference in the ability of BNO 1030 to modulate cytokine secretion induced by IL-1β (Fig. 2B) was noted (data not shown). As shown in Fig. 2, LPS and/or IL-1β-induced level of IL-8 were inhibited by BNO 1030 in A549 cells in a dose-dependent manner. The IL-8 response of respiratory A549 cells stimulated by LPS was significantly reduced by a treatment with the extract at a concentration as low as 1 μg/ml ($p = 0.037$; $F = 8.9$). Because the basal level of IL-8 varied among the experiments in A549 cells (542–1180 pg/ml) results are presented as relative percentage to LPS or IL-1β controls (Fig. 2A and B). Basal level of IL-8 was 50.17 ± 13.5% in LPS-stimulated experiments. BNO 1030 at

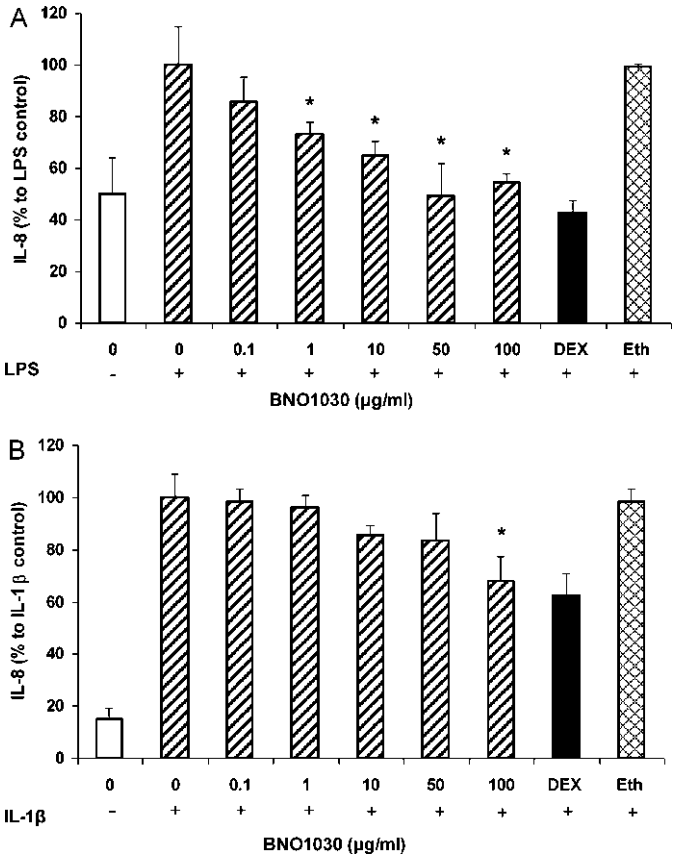

Fig. 2. Effect of BNO 1030 on LPS (A) or IL-1β (B)-induced release of IL-8 in A549 cells. Respiratory A549 cells were stimulated with LPS (100 μg/ml) or IL-1β (50 ng/ml) in the presence of indicated concentrations of BNO 1030 for 18 h. Levels of IL-8 in supernatants were determined by ELISA, normalized to protein concentration and relative values to stimulated controls are expressed as means ± SD in percentage. Assays were performed in duplicate of three (A) or two (B) different experiments (*signifies $p < 0.05$ of observed effect vs. LPS or IL-1β stimulated control). DEX (10^{-7} M) was used as positive control. The solvent 0.5% ethanol alone did not affect the release of IL-8. IC_{50} value derived for IL-8 suppression by BNO 1030 in LPS stimulated A549 cells was 5.7 ± 5.6 μg/ml (A).

50 μg/ml and 100 μg/ml concentration suppressed the activation of IL-8 production completely to the basal levels (49.3% and 54.4%) in three independent experiments ($p = 0.027$; $F = 3.2$ and $p = 0.016$; $F = 18.3$). In LPS-stimulated A549 cells, the IC_{50} of BNO 1030 was determined as 5.7 ± 3.6 μg/ml.

In IL-1β-stimulated A549 cells the highest concentration tested (100 μg/ml) led to an inhibition of IL-8 secretion by $37.7 \pm 4.1\%$ ($p = 0.036$; $F = 2.4$) from 3353.5 ± 292 pg/ml to 2275.5 ± 245 pg/ml. The basal level of IL-8 protein in A549 cells was 489.5 ± 111 pg/ml. This concentration represents $15 \pm 4.2\%$ of the IL-1β-stimulated values. DEX at a dose of 10^{-7} M was used as positive control in each experiment. DEX suppressed the effect of LPS completely to the basal level of A549 cells (Fig. 2A) and inhibited IL-1β-induced secretion of IL-8 by $44.0 \pm 4.2\%$ to 2093 ± 267.3 pg/ml (Fig. 2B). The solvent 0.5% ethanol did not affect the release of IL-8 in LPS- and/or IL-1β-stimulated cells. The releases of IL-8 were 99.1% and 98% from the stimulated controls, respectively.

3.3. BNO 1030 inhibited IL-1β-induced production of hBD-2 in pulmonary A549 cells

Lung epithelial cells have been reported to participate in innate host defense by expressing antimicrobial peptide hBD-2 in response to inflammatory stimuli such as LPS and proinflammatory cytokines (MacRedmond et al., 2005). IL-1β-activated A549 cells were incubated with various concentrations (0.01–100 μg/ml) of BNO 1030. Changes in hBD-2 secretion were comparable to that observed for IL-8. A dose dependent inhibition of hBD-2 secretion could be demonstrated by BNO 1030 (Fig. 3). Significant ($p < 0.05$; $F = 2.1$) suppression of inducible hBD-2 was detected at a concentration as low as 0.1 μg/ml of BNO 1030 (222.5 ± 20.5 pg/ml) compared to IL-1β-induced control (307 ± 29.7 pg/ml). BNO 1030 at concentration of 0.7 ± 0.1 μg/ml inhibited the induction of hBD-2 by 50%. The inhibitory effect of BNO 1030 between 10 μg/ml and 100 μg/ml is approaching a plateau. BNO 1030 at a concentration of 100 μg/ml suppressed the IL-1β-induced hBD-2 secretion by $91.8 \pm 15.6\%$ to 65.5 ± 20.5 pg/ml ($p < 0.01$; $F = 14.5$). Positive control DEX (10^{-7} M) diminished the effect of IL-1β to 51 ± 5.6 pg/ml hBD-2. In unstimulated control 41.4 ± 1.4 pg/ml hBD-2 was detected.

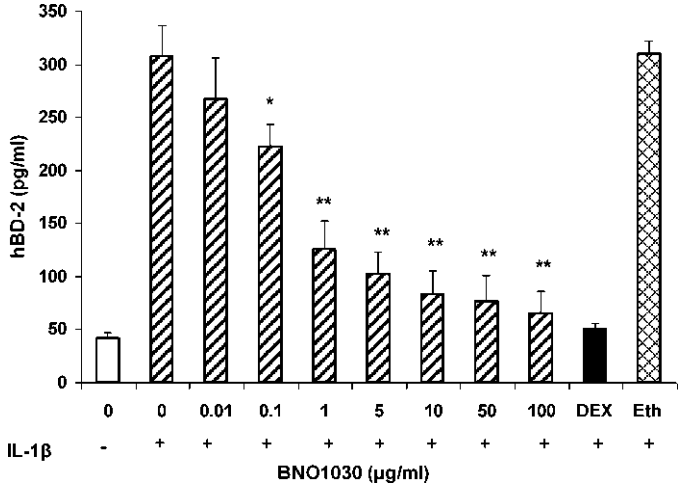

Fig. 3. hBD-2 response to BNO 1030 in cytokine-stimulated A549 cells. A549 cells were stimulated with 50 ng/ml IL-1β in the presence of indicated concentrations of BNO 1030 for 24 h. Levels of hBD-2 protein were determined by ELISA in the culture supernatants. Data are expressed as mean ± SD from duplicate of two independent experiments. Values were compared between control cytokine-stimulated cells and stimulated cells treated with indicated concentrations of extract. $^{*}p < 0.05$; $^{**}p < 0.01$. IL-1β induced level of hBD-2 (307 ± 29.7) pg/ml was inhibited by DEX (10^{-7} M) to 51 ± 5.6 pg/ml. In unstimulated control 41 ± 1.4 pg/ml hBD-2 was detected. The solvent 0.5% ethanol alone did not affect the release of hBD-2. The IC_{50} of BNO 1030 evaluated from dose-response curves was 0.7 ± 0.1 μg/ml.

4. Discussion

In bacterial infectious diseases, host cells should first recognize and respond to bacterial cell surface components via the innate immune system and develop inflammatory and immunological reactions (Vroling et al., 2008). The TLR family was shown to play a key role in signalling of host cells in response to bacterial cell surface components (Kopp and Medzhitov, 1999). Expression of TLR4 on respiratory epithelium allows rapid activation of host defense by pathogens, leading to induction of inflammatory mediators and anti-microbial peptides, including IL-8 and hBD-2 (MacRedmond et al., 2005).

Plants produce a variety of secondary metabolites that have long been of medicinal interest to man. In recent years these are being used, either directly as precursors or as lead compounds, in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. Plain extracts, single compounds and/or fractions of characteristic substances such as polyphenols, gallotannins or epigallocatechin gallate originating from plants are known to possess anti-inflammatory properties (Erdelyi et al., 2005; Kim et al., 2006). BNO 1030 is an extract of seven different herbal drugs, most of them contain phenolic compounds such as flavonoids, tannins and phenolic acids which have anti-inflammatory as well as anti-oxidative properties (Hu and Kitts, 2003; Erdelyi et al., 2005; Kim et al., 2006).

In this study we investigated the impact of BNO 1030 on the secretion of the CXC chemokine IL-8 and of the microbial peptide hBD-2 in A549 airway epithelial cells. Epithelial cells are not a simple barrier against micro-organisms, they also actively modulate inflammatory responses by the generating cytokines and chemokines, which attract leucocytes to the sites of inflammation (Kunkel et al., 1991; Strieter et al., 2002; Shaykhiev and Bals, 2007; Vroling et al., 2008).

BNO1030 exerted very low toxicity on A549 cells. The membrane of cells was intact up to 2000 μg/ml concentration of BNO1030 as detected by flow cytometry using PI. In addition, the cell growth was significantly affected at concentration above the 500 μg/ml with IC_{50} being of 678 μg/ml herbal BNO 1030. Pre-stimulation with LPS lowered the inhibitory effect of BNO 1030. IC_{50} value was increased to 796 μg/ml, however this effect was non significant (Fig. 1).

We could show that at low non-cytotoxic concentrations BNO 1030 significantly diminished the effect of LPS or IL-1β on IL-8 secretion (Fig. 2). Because of controversial results regarding the constitutive expression of TLR4 on the surface of A549 cells (Tsutsumi-Ishii and Nagaoka, 2003; MacRedmond et al., 2005), we first examined its expression on the surface on A549 cells. In our experiments, the lung epithelial A549 cells expressed TLR4 on their surface (data not shown). Despite TLR4 expression on A549 cells, a higher dose of bacterial LPS (100 μg/ml) was needed to activate the cells compared to the proinflammatory cytokine IL-1β (50 ng/ml). In the A549 cells activated by IL-1β, protein level increased about 7-fold in comparison to its basal level, whereas the LPS-activated cells produced about 2-fold higher protein concentrations. This was consistent with previous reports in which IL-1β was the most potent stimulus for IL-8 induction in airway epithelial cells (Strieter et al., 2002; Kim et al., 2006). BNO 1030 at concentration of 50 μg/ml diminished the LPS-activated level of IL-8 to its basal unstimulated level. However the suppression of IL-1β-induced IL-8 by BNO 1030 was lower (37.7%). A possible reason for this lower activity of BNO 1030 could be the high concentration of induced IL-8 after IL-1β

stimulation, because the positive control 10^{-7} M DEX inhibited the level of IL-8 comparably. These results were consistent with finding that the 8–9-fold activation by IL-1β of A549 cells was repressed 30–40% by DEX (Newton et al., 1998).

A549 cells secreted IL-8 without any cytokine or LPS stimulation. Therefore a higher variation in the basal level of protein in A549 cells and between the experiments is possible as reported by others (Pelletier et al., 2002). This could be explained by the observation that A549 cells express protease activated receptors (PAR)-1 to PAR-4. Trypsin is a PAR-2/PAR-4 agonist which is commonly used for the detachment of cells and induced IL-8 in these cells (Asokananthan et al., 2002). PARs could be responsible for the variability in the basal level of IL-8 on A549 cells. To eliminate a possible endotoxin contamination of BNO 1030 we used polymyxin B in experiments by IL-1β primed A549 cells, because this LPS-neutralizing antibiotic specifically inhibits LPS signalling, whereas it has no effects on response to Il-1β (Bäckhed et al., 2002). Results from the present study are consistent with these observations.

As shown in Fig. 3, BNO 1030 caused suppression of the inducible hBD-2 on A549 cells by IL-1β. This effect was significant already at a dose of 0.1 μg/ml of BNO 1030. In addition, BNO 1030 nearly completely diminished the activating effect of IL-1β on hBD-2 induction to its basal level on resting A549 cells.

Defensins and chemokines are an essential part of the immune response. Association exists between the innate and acquired immune system on the defensin-chemokine level. Recent studies have shown that the levels of hBD-2 strongly correlated with those of increased IL-8 from airway epithelial cells *in vitro* and in tonsillar diseases (van Wetering et al., 1997; Meyer et al., 2006). Our results are consistent with these reports, because both inflammatory mediators, the hBD-2 and chemokine IL-8 were suppressed by BNO 1030. Correlations between hBD-2 and IL-8 are biologically relevant because both inhibit Th2 cytokines and thereby enhance the immune response of the Th1 system (Nomura et al., 2003). Defensins and inflammatory markers were expressed at low levels in healthy individuals and at high levels in subjects with acute cold (Dauletbaev et al., 2002).

The effect of BNO 1030 is probably caused by the positive interaction of several active compounds (e.g. polysaccharides, flavonoids, tannins) of single herbal extracts on neutrophil-mediated inflammatory processes (Hougee et al., 2005; Kim et al., 2006; Deters et al., 2010). Several reports suggest that the regulation of IL-8 production and the activation of hBD-2 by bacteria or cytokine IL-1β is regulated in an NF-κB-dependent manner (Tsutsumi-Ishii and Nagaoka, 2003). NF-κB is induced by IL-1β in A549 cells and induce transcription of several genes involved in innate immune responses (Strieter et al., 2002). Recently it has been reported that natural products inhibited the IL-8 production and a large number of plant-derived substances have been evaluated as possible inhibitors of the NF-κB pathway (Nam et al., 2006). Glucocorticoid repressed numerous proinflammatory genes including IL-8 and NF-κB and κB-related transcriptions have been proposed as targets for glucocorticoid repression (Kwon et al., 1994). In our studies extract repressed the IL-8 secretion in LPS and IL-1β-primed A549 cells to a similar extent as DEX (Fig. 2). Additional studies on NF-κB pathways are necessary to elucidate its modulating effect in inflammatory processes.

5. Conclusion

Our results provide evidence that the herbal extract BNO 1030 has the potential to reduce the secretion of key inflammatory chemokine IL-8 and antimicrobial peptide hBD-2 in respiratory epithelial cells *in vitro*. These finding substantiate the use of BNO 1030 as a phytotherapetic product derived from traditional medicinal herbs for the treatment of inflammatory disorders particularly those affecting the respiratory tract. In addition, these results give a partial scientific explanation for the long time use of BNO 1030 and are in good accordance with the traditional use of herbs consisted.

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