



Cysteinyl leukotriene receptor antagonist MK-571 alters bronchoalveolar lavage fluid proteome in a mouse asthma model

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Abstract

Cysteinyl leukotriene receptor type 1 (leukotriene CysLT₁ receptor) antagonist is one of the most effective anti-inflammatory agents for asthma. The spectrum of protein targets that can be regulated by leukotriene CysLT₁ receptor antagonist in asthma is not fully understood. The present study tried to identify novel protein targets of a selective leukotriene CysLT₁ receptor antagonist MK-571 in allergic airway inflammation by analyzing the proteome of mouse bronchoalveolar lavage fluid. BALB/c mice sensitized and challenged with ovalbumin showed increased pulmonary inflammatory cell infiltration, airway mucus production and serum ovalbumin-specific IgE level. MK-571 inhibited all these allergic airway inflammation endpoints. Lavage fluid proteins were resolved by two-dimensional gel electrophoresis and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The level of fourteen bronchoalveolar lavage fluid protein spots was markedly altered by MK-571. A family of chitinases (Ym1, Ym2 and acidic mammalian chitinase), lungkine, surfactant protein-D and γ -actin have been found for the first time to be down-regulated by leukotriene CysLT₁ receptor antagonist in mouse allergic airways. Some of the down-regulatory effects were confirmed with reverse transcription-polymerase chain reaction analyses. Taken together, we have identified novel protein targets that can be regulated by leukotriene CysLT₁ receptor antagonist in mouse allergic airway inflammation, and our findings reveal additional pharmacological actions of leukotriene CysLT₁ receptor antagonist in the treatment of asthma.

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1. Introduction

Allergic asthma is a chronic lung disease characterized by airway inflammation, mucus hypersecretion and bronchial hyperresponsiveness (Busse and Lemanske, 2006). Cysteinyl leukotrienes (e.g. LTC₄, LTD₄ and LTE₄) play a critical role in allergic airway inflammation. Cysteinyl leukotrienes are potent bioactive lipid mediators and are produced primarily by eosinophils, mast cells, basophils and alveolar macrophages. Cysteinyl leukotrienes mediate various aspects of allergic airway inflammation including airway hyperresponsiveness, mucus hypersecretion, eosinophil infiltration and enhanced eosinophil

survival, Th2 cytokine (e.g. IL-4, IL-13 and IL-5) production, collagen and macrophage-derived fibroblast growth factor production for airway remodeling, and microvascular leakiness (Panettieri et al., 1998; Fregonese et al., 2002; Vargaftig and Singer, 2003). While all the cysteinyl leukotrienes have been shown to bind to the seven-transmembrane leukotriene CysLT₁ receptor and leukotriene CysLT₂ receptor, most of the known actions are mediated through leukotriene CysLT₁ receptor (Kanaoka and Boyce, 2004; Ogawa and Calhoun, 2006). Indeed, leukotriene CysLT₁ receptor antagonist is one of the most effective anti-inflammatory agents for asthma. Leukotriene CysLT₁ receptor antagonist effectively reduces airway inflammation and suppresses production of pro-inflammatory proteins like IL-4, IL-5, and IL-13 (Busse and Kraft, 2005; Ogawa and Calhoun, 2006). At present, the full spectrum of protein regulation by leukotriene CysLT₁ receptor antagonist in asthma is not understood. Using proteomics technology, it is now feasible to

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study global protein expression in allergic airways in response to leukotriene CysLT₁ receptor antagonist therapy.

There are increasing numbers of reports using the proteomics approach to study asthma (Signor et al., 2004; Candiano et al., 2005; Jeong et al., 2005; Wu et al., 2005; Zhao et al., 2005); however, there is no proteomics study of bronchoalveolar lavage fluid from leukotriene CysLT₁ receptor antagonist-treated asthmatic mice. The present study investigated the pharmacological effect of a leukotriene CysLT₁ receptor antagonist MK-571 (3-((3-(2-(7-chloro-2-quinoliny)ethenyl)phenyl)((3-(dimethylamino-3-oxopropyl)thio)-methyl)thio)propanoic acid) (Jones et al., 1989; Blain and Sirois, 2000) on global protein expression in lavage fluid from a mouse asthma model. Ovalbumin-sensitized and -challenged mice developed pulmonary eosinophilia, mucus hypersecretion and elevation of serum ovalbumin-specific IgE levels. Lavage fluids from mice with or without MK-571 treatment were resolved by two-dimensional gel electrophoresis, and proteins with at least 2-fold change in optical density were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. MK-571 markedly altered multiple lavage fluid protein expression in allergic airway inflammation. In particular, leukotriene CysLT₁ receptor antagonist was found for the first time to block the elevated lavage fluid levels of Ym1, Ym2, acidic mammalian chitinase, and lungkine in allergic airways.

2. Materials and methods

2.1. Mouse asthma model and drug treatment

All animal studies were performed in accordance with the guidelines of Institutional Animal Care and Use Committee of the National University of Singapore. Male BALB/c mice 5–7 weeks of age (Interfauna, East Yorkshire, UK) were sensitized by intraperitoneal injections of 20 µg of ovalbumin (Grade V, Sigma, St. Louis, MO) together with 4 mg of Al(OH)₃ suspended in 0.1 ml saline on days 0 and 14 as described (Duan et al., 2005). On days 18, 19 and 20, animals were challenged with 1% ovalbumin aerosol for 20 min. Control mice were challenged with saline aerosol. MK-571 (Cayman Chemical, Ann Arbor, MI) was dissolved in sterile saline and was administered intraperitoneally at 25 or 50 mg/kg 1 h before every ovalbumin aerosol challenge. Mice were sacrificed by cervical dislocation 24 h after the last ovalbumin aerosol challenge. Bronchoalveolar lavage fluid was collected as described (Duan et al., 2005). Lavage fluid cells were pelleted for total and differential cell counts, and the supernatants were prepared for 2-dimensional gel electrophoresis. Lung tissues were processed for histological evaluation of pulmonary cell infiltration (hematoxylin and eosin) and mucus hypersecretion (periodic acid–Schiff stain). Blood was collected by cardiac puncture for serum IgE and IgG measurements.

2.2. Proteomics of bronchoalveolar lavage fluid

Lavage fluid proteins were precipitated in 10% trichloroacetic acid and washed with ice-cold acetone. The protein pellet was air-dried and dissolved in sample buffer containing 8 M

urea, 2 M thiourea, 4% 3-(3-cholamidopropyl-dimethylammonio)-1-propane sulfonate, 0.8% pharmalyte, 65 mM dithiothreitol, protease inhibitor cocktail, 5 µg/ml DNase I and 20 µg/ml RNase A. 2-DE was performed as described (Zhao et al., 2005). The first dimension was run using Immobiline Drystrip (18 cm, pH 4–7, Amersham Biosciences, San Francisco, CA) on an IPGphor (Amersham Biosciences). The second dimension was carried out in an Ettan™ DALT six vertical system (Amersham Biosciences). Gels were silver stained, scanned with a GS-800 densitometer (Bio-Rad, Hercules, CA), and analyzed using the PDQuest software (version 7.2, Bio-Rad) for protein spot detection, quantitation, and matching. Each spot on the gel was given an integrated optical density value by the software program, which was normalized based on the total amount of valid spots on that gel. Thus each spot was expressed as parts per million of the total integrated optical density of all valid spots. Matched protein spots with statistically significant optical density changes between control and MK-571-treated groups were selected for trypsin digestion. Peptide mass fingerprint maps of tryptic peptides were generated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a Voyager DE-STR Biospectrometry work station mass spectrometer (Applied Biosystems, Foster City, CA). Peptide masses were searched against the NCBI database with the Mascot software (<http://www.matrixscience.com>) and the MS-Fit program (<http://prospector.ucsf.edu>). Proteins with a minimum of four matching peptides and the sequence coverage exceeding 20% with the matched proteins, and with a probability score >75 were considered positive.

2.3. Reverse transcription-polymerase chain reaction

Total mRNA from lung tissues were extracted using a RNA isolation kit (Ambion, Austin, TX). First strand cDNA was synthesized from 1 µg RNA using oligo(dT)₁₅ primers and 10 U AMV reverse transcriptase. Polymerase chain reaction was performed in a GeneAmp PCR system 2700 thermal cycler (Applied Biosystems). The following sets of primers were used: lungkine, forward, 5'-TATTCCCGCGTTAGTCTGGTG-3' and reverse, 5'-GCCCATAGTGGAGTGGGATAAG-3'; Ym1, forward, 5'-CTGGAATTGGTGCCCCTACA-3' and reverse, 5'-CAAGCATGGTGGTTTACAGGA-3'; Ym2, forward, 5'-CAGAACCGTCAGACATTCATTA-3' and reverse, 5'-ATGGTCCTTCCAGTAGGTAATA-3'; acidic mammalian chitinase, forward, 5'-TGGGTCTGGGCTACTATG-3' and reverse, 5'-GCTTGACAATGCTGCTGGTA-3'; surfactant protein-D, forward, 5'-CTCTCTGCACACTCCATCCA-3' and reverse, 5'-CAAGGCCACTGTTCCCTTAGC-3'; and β-actin, forward, 5'-TCATGAAGTGTGACGTTGACATCCGT-3' and reverse, 5'-CCTAGAAGCATTGCGGTGACGATG-3'. Polymerase chain reaction products were run in a 1.5% agarose gel and visualized under UV light.

2.4. Statistical analysis

Changes in optical density of all matched protein spots between groups were subjected to ANOVA followed by Tukey

test using SPSS software (Chicago, IL). Data were expressed as mean $\pm$ S.E.M. The critical level for significance was set at $P < 0.05$.

3. Results

3.1. MK-571 inhibits airway inflammation in mice

Ovalbumin-sensitized and ovalbumin-challenged mice showed markedly higher total cell and eosinophil counts as

compared to ovalbumin-sensitized and saline-challenged control mice. Lymphocyte count was also significantly elevated in ovalbumin-sensitized and ovalbumin-challenged mice. MK-571 (25 and 50 mg/kg) substantially reduced the total cell number and eosinophils recovered in lavage fluid as compared to vehicle control (Fig. 1A). Ovalbumin aerosol challenge induced marked infiltration of inflammatory cells, especially eosinophils, into the lamina propria, and perivascular and peribronchiolar areas as compared to saline aerosol challenge.

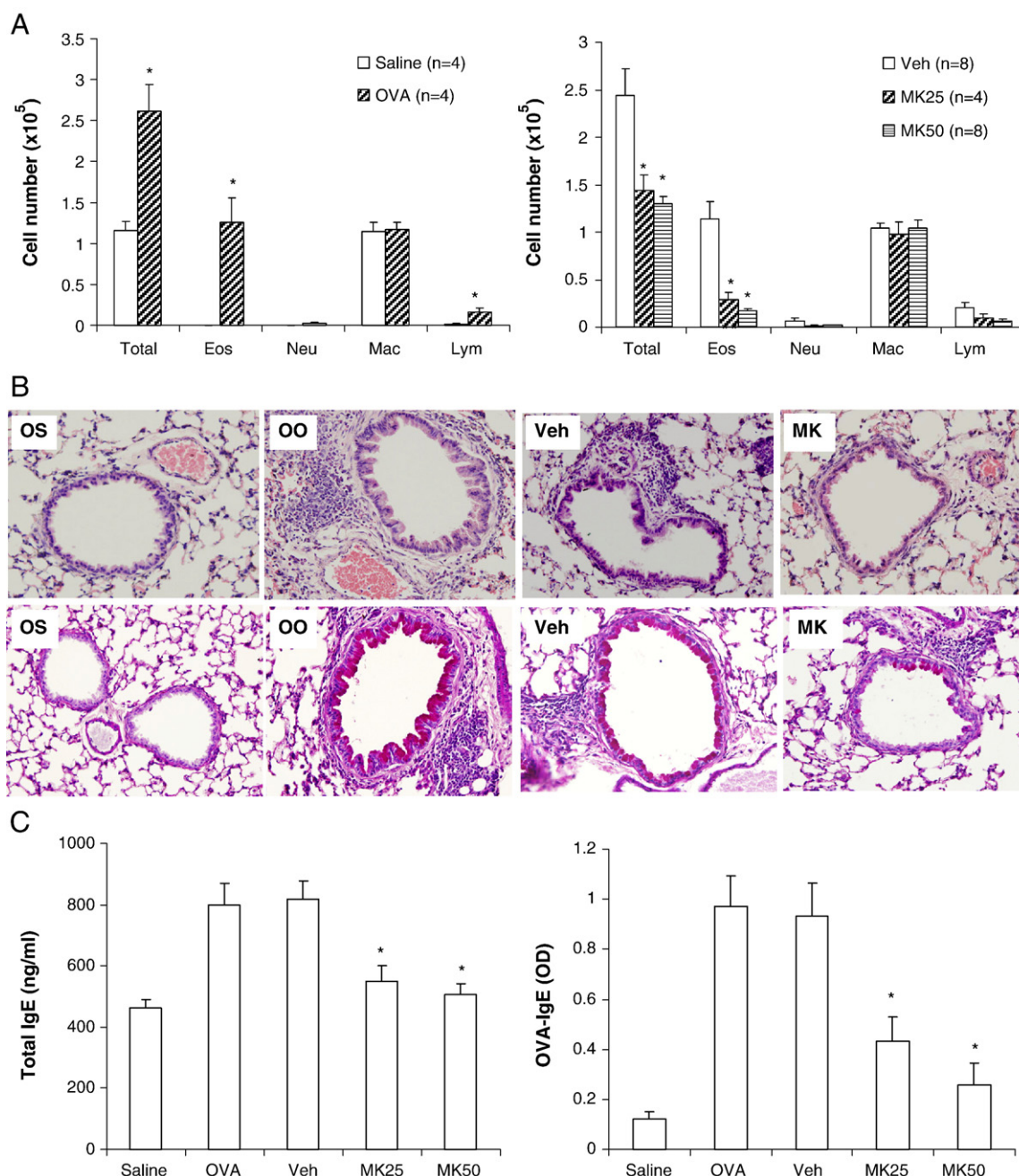


Fig. 1. Anti-inflammatory effects of leukotriene CysLT₁ receptor antagonist MK-571 on mouse allergic airway inflammation. (A) Bronchoalveolar lavage fluids were collected 24 h after the last saline or ovalbumin (OVA) aerosol challenge. Total and differential cell counts were performed. Mice were treated with either saline vehicle (Veh) or MK-571 (MK25, 25 mg/kg or MK50, 50 mg/kg) intraperitoneally prior to every ovalbumin aerosol challenge. Eos, eosinophil; Neu, neutrophil; Mac, macrophage; Lym, lymphocyte. (B) Histological examination of lung tissue cell infiltration (upper panels) and mucus production (lower panels) 24 h after the last saline (OS) or ovalbumin (OO) challenge in the presence (MK) and absence (Veh) of MK-571 (50 mg/kg) was performed under a microscope at 400X magnification. (C) Serum levels of total IgE and ovalbumin-specific IgE were analyzed using ELISA ($n=4$). *Significant difference from vehicle group, $P < 0.05$.

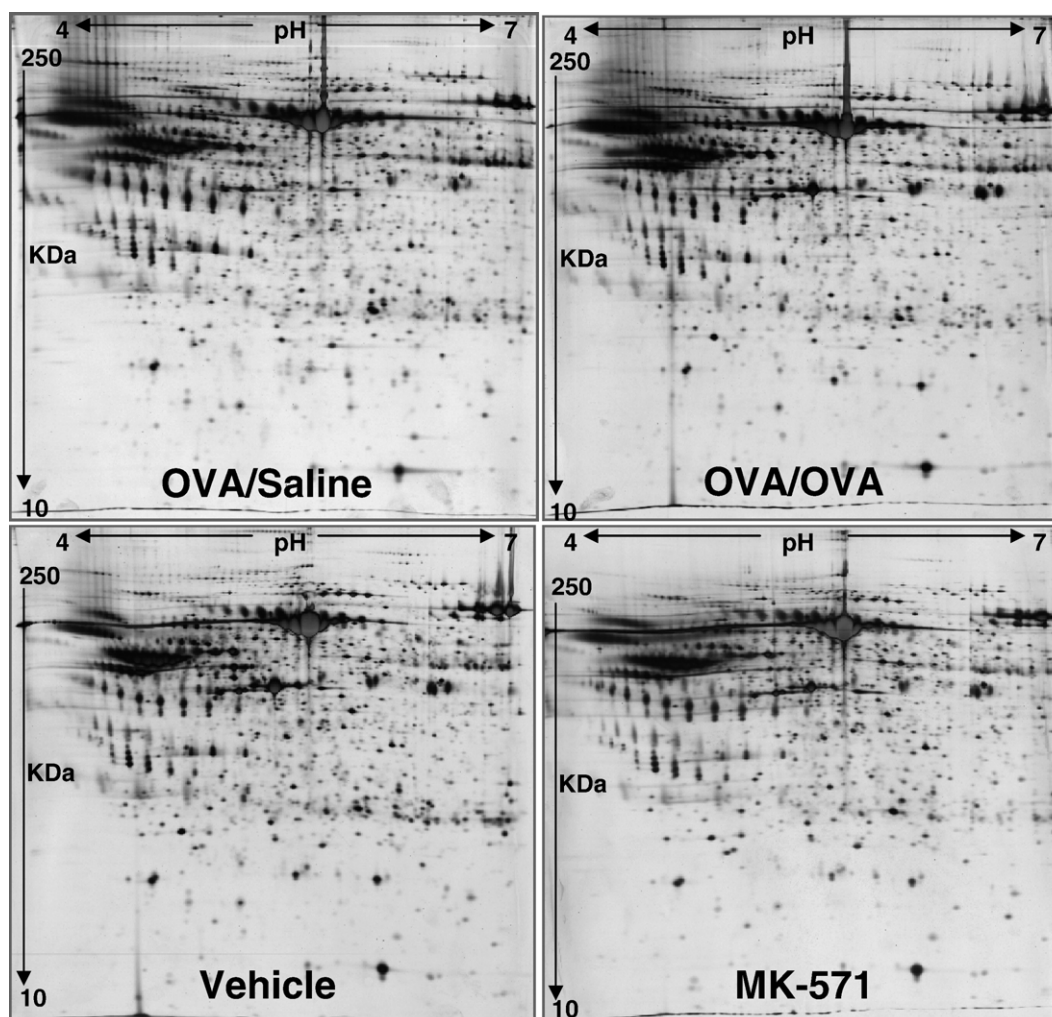


Fig. 2. Representative 2-dimensional gels of bronchoalveolar lavage fluid samples corresponding to ovalbumin-sensitized and saline-challenged (OVA/saline, $n=4$), ovalbumin-sensitized and ovalbumin-challenged (OVA/OVA, $n=4$), vehicle-treated ($n=8$), and 50 mg/kg MK-571-treated ($n=8$). Lavage fluids were separated by 2-dimensional gel electrophoresis using IPG at pH 4–7 12% SDS-PAGE. Gels were stained with silver nitrate, scanned using a GS-800 calibrated imaging densitometer (Bio-Rad), and analyzed using PD-QUEST (Version 7.2) software (Bio-Rad) for protein spot detection, quantitation and matching.

MK-571 attenuated eosinophil-rich leukocyte infiltration compared with vehicle control. On the other hand, ovalbumin-sensitized and ovalbumin-challenged mice, but not ovalbumin-sensitized and saline-challenged mice, developed marked goblet cell hyperplasia and mucus hypersecretion within the bronchi in the lung. The ovalbumin-induced mucus secretion was significantly abated by MK-571 compared to vehicle control (Fig. 1B). Substantial elevation in total IgE and ovalbumin-specific IgE was observed in serum from ovalbumin-sensitized and ovalbumin-challenged mice as compared with ovalbumin-sensitized and saline-challenged mice. MK-571 significantly ($P<0.05$) lowered total IgE and ovalbumin-specific IgE levels (Fig. 1C).

3.2. MK-571 alters mouse bronchoalveolar lavage fluid proteome

A total of 24 lavage fluid samples from ovalbumin-sensitized and saline-challenged ($n=4$), ovalbumin-sensitized and ovalbumin-challenged ($n=4$), and ovalbumin-sensitized and oval-

bumin-challenged treated with saline vehicle ($n=8$) and 50 mg/kg MK-571 ($n=8$) were resolved by 2-dimensional gel electrophoresis. Similar protein patterns were observed on 2-dimensional gels for all the groups studied (Fig. 2). Following image analysis, about 450 spots were consistently detected and matched on all gels of each group, and were subjected to differential protein expression analysis. MK-571 treatment markedly altered the expression level of a total of 14 lavage fluid protein species ($P<0.05$) in allergic airway inflammation, and they were summarized in Table 1. The location of the 14 identified protein spots were shown in a representative gel (Fig. 3). Direct comparison of the optical densities of protein spots among treatment groups was illustrated in Fig. 4. Several proteins existed in multiple spots (e.g. Ym1 and lungkine) of similar molecular weights of different pIs, which may be a result of posttranslational modifications such as glycosylation and phosphorylation. We have for the first time demonstrated that MK-571 was able to suppress the elevated lavage fluid levels of a family of chitinases, Ym1 (spot nos. 1–3), Ym2 (spot no. 4) and acidic mammalian chitinase (spot no. 5) (Donnelly and

Table 1
Leukotriene CysLT₁ receptor antagonist MK-571 alters bronchoalveolar lavage fluid protein expression in allergic airway inflammation

No.	Name	Accession ID	Score ^a	Matched peptides	MK-571 (50 mg/kg) fold-change
1	Ym1	gi285015	122	HLFSVLVK, DGYTGENSEPLYK FGPAPFSAMVSTPQNR, DNNLGGAVVWPLDMDDFSGSFCHQR	−2.4
2	Ym1	gi285015	78	QIFIQSVIR, DGYTGENSEPLYK FGPAPFSAMVSTPQNR, LLLTSTGAGIIDVIKSGTR	−2.2
3	Ym1	gi285015	242	HLFSVLVK, DGYTGENSEPLYK FGPAPFSAMVSTPQNR, DNNLGGAVVWPLDMDDFSGSFCHQR	−2.1
4	Ym2	gi22123907	95	TLLAIGGWK, AFEEESTLNHIPR LIVGFPAYGHTFILSDPSK, DNNLGGAVVWPLDMDDFSGSFCHQGR	−2.5
5	Acidic mammalian chitinase	gi37999744	190	FPLTSTLNK, LIVGFPEYGHTFILR SGATEVWDASQEVPIYAYK, YPTETGSNAYLNVYVMNYWK	−3.6
6	Lungkine	gi5031129	128	IFPFPIR, ATVGPIITNR HFADLAHNDR, HGADLAHNDRNFLR	−2.2
7	Lungkine	gi5031129	78	IFPFPIR, ATVGPIITNR HFADLAHNDR, HGADLAHNDRNFLR	−2.5
8	Lungkine	gi5031129	130	IFPFPIR, ATVGPIITMR HFADLAHNDR, NIMVIFETIYCNR	−2.8
9	Lungkine	gi5031129	86	IFPFPIR, ATVGPIITMR HFADLAHNDR, NIMVIFETIYCNR	−3.5
10	Surfactant protein-D	gi6524992	108	AALFPDGR, AAFLSMTDVGTEGK SVPNTCTLVMCSPTEGLPGR, GEKGDPLPGPMGLSGLQGPTGPVGP	−2.4
11	Surfactant protein-D	gi6524992	260	AALFPDGR, AAFLSMTDVGTEGK SVPNTCTLVMCSPTEGLPGR, GEKGDPLPGPMGLSGLQGPTGPVGP	−3.1
12	γ-actin	gi809561	355	AGFAGDDAPR, SYELPDGQVITIGMER KDLYANTVLSGGTTMYPGIADR, TTGIVMDSGDGVTHTVPIYEGYALPHAILR	−2.4
13	Unidentified	/	/	Not detectable	−4
14	Unidentified	/	/	Not detectable	−2.4

^a Score is $-10 \times \log(P)$, where P is the probability that the observed match is a random event. Protein score of greater than 73 is considered significant ($P < 0.05$).

Barnes, 2004), lungkine (spot nos. 6–9) (Chen et al., 2001), surfactant protein-D (spot nos. 10 and 11) (Hackzku et al., 2004), γ-actin (spot no. 12) (Khaitlina, 2001), and two un-

identifiable protein spots (spots no. 13 and 14). Consistent with our previous report (Zhao et al., 2005), gob-5 protein, polymeric immunoglobulin receptor, α-1 antitrypsin, heptoglobin, vitamin

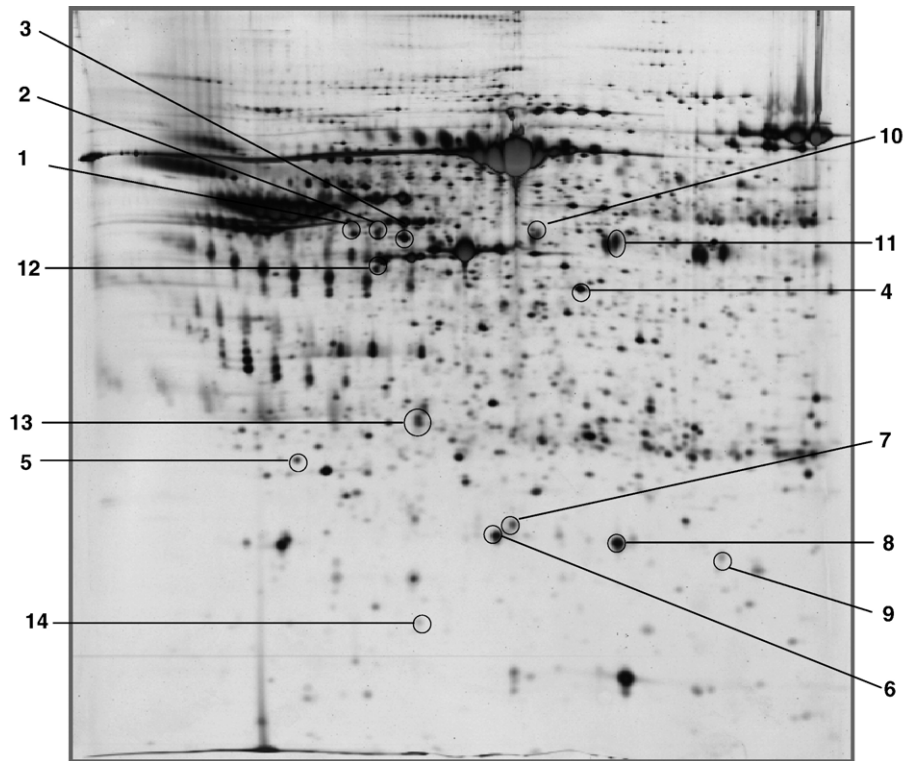


Fig. 3. A representative gel image indicates the locations of all 14 bronchoalveolar lavage fluid protein spots that have been shown to be significantly altered by leukotriene CysLT₁ receptor antagonist MK-571. Numbers correspond to the spot identification numbers listed in Table 1.

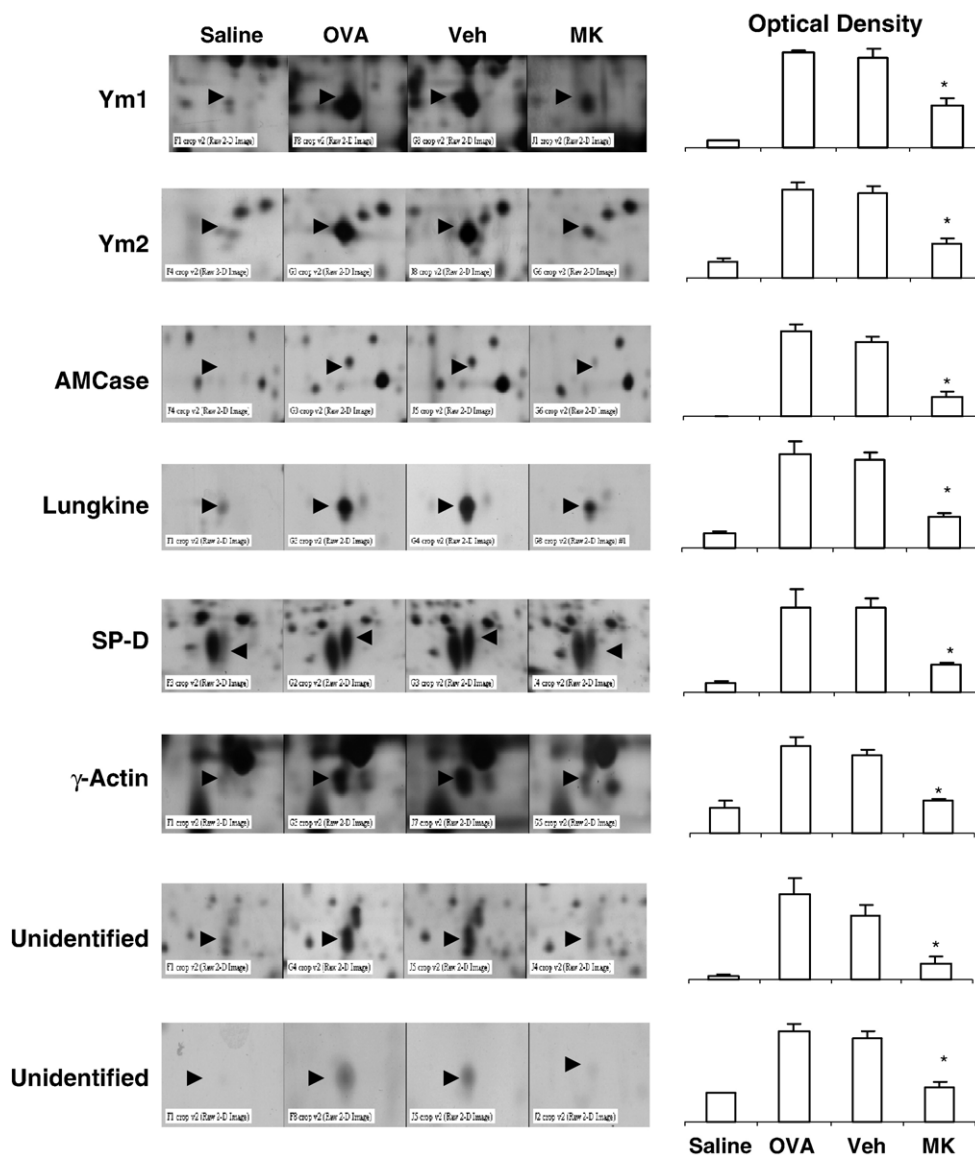


Fig. 4. Proteins significantly altered by leukotriene CysLT₁ receptor antagonist MK-571 in asthma mouse bronchoalveolar lavage fluid. The left panel shows spots of differentially expressed proteins in randomly selected 2-dimensional gels. The right panel shows the corresponding bar charts of spot intensities (Saline, $n=4$; ovalbumin (OVA), $n=8$; vehicle (Veh), $n=8$; 50 mg/kg MK-571 (MK), $n=8$). AMCase, acidic mammalian chitinase; SP-D, surfactant protein-D.

D-binding protein and immunoglobulin light chain variable region were up-regulated in the mouse asthma model. However, MK-571 did not alter the expression level of these proteins.

3.3. Reverse transcription-polymerase chain reaction

To verify the 2-dimensional gel electrophoresis findings, we conducted reverse transcription-polymerase chain reaction analysis on selected protein targets. In line with the proteomics observation, ovalbumin aerosol challenge clearly elevated mRNA expression of a family of chitinases (Ym1, Ym2 and acidic mammalian chitinase), lungkine and surfactant protein-D; and MK-571 treatment markedly suppressed mRNA expression of the above gene targets in allergic airway inflammation (Fig. 5). Gene expression of Th2 cytokine IL-13 was strongly induced in allergic airways and was completely abolished by MK-571.

4. Discussion

Leukotriene CysLT₁ receptor antagonist is one of the most effective anti-inflammatory agents for asthma. Cysteinyl leukotrienes mediate various aspects of allergic airway inflammation including airway hyperresponsiveness, mucus hypersecretion, eosinophil infiltration and enhanced eosinophil survival, Th2 cytokine (e.g. IL-4, IL-13 and IL-5) production, collagen and macrophage-derived fibroblast growth factor production for airway remodeling, and microvascular leakiness (Kanaoka and Boyce, 2004; Ogawa and Calhoun, 2006). However, the full spectrum of protein targets that can be regulated by leukotriene CysLT₁ receptor antagonist is not understood. To our knowledge, this is the first pharmacoproteomics study of leukotriene CysLT₁ receptor antagonist in allergic airway inflammation. We applied proteomics tools to

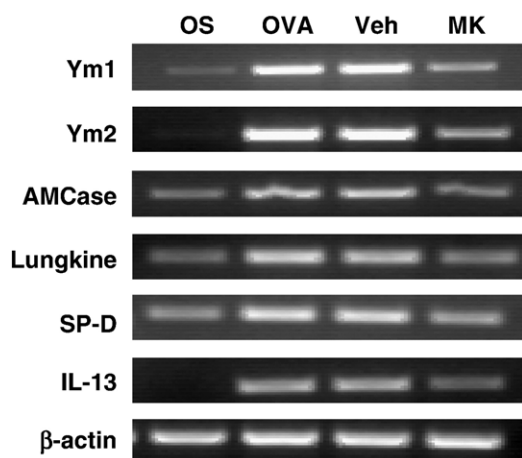


Fig. 5. Effects of leukotriene CysLT₁ receptor antagonist MK-571 on mRNA expression of acidic mammalian chitinase (AMCase), Ym1, Ym2, lungkine, surfactant protein-D (SP-D) and IL-13 in lung tissues from mouse asthma model. Total mRNA was extracted using RNA isolation kit and the polymerase chain reaction products were separated in a 2% agarose gel visualized under UV light. The experiments were repeated for four times with similar pattern of results. OS, ovalbumin-sensitized and saline-challenged; OVA, ovalbumin-sensitized and ovalbumin-challenged; vehicle, saline; MK, MK-571. β -actin was used as a control.

compare protein profiles of bronchoalveolar lavage fluids from mice treated with or without a leukotriene CysLT₁ receptor antagonist MK-571 in order to discover novel protein targets in the treatment of allergic asthma.

Chitinases belong to the family 18 of glycosyl hydrolases that can cleave β -glycosidic linkages between *N*-acetylglucosamine residues of chitin molecules (Donnelly and Barnes, 2004). At least seven chitinases are expressed in mammals, which include chitotriosidase, acidic mammalian chitinase, human cartilage glycoprotein-39, porcine gp38 K, mouse brp39, and mouse Ym1 and Ym2 (Nio et al., 2004). Only chitotriosidase and acidic mammalian chitinase possess chitinolytic activity (Boot et al., 2001). A recent study demonstrated an increase in acidic mammalian chitinase level in a mouse asthma model and in asthmatic subjects, and an anti-inflammatory effect in a mouse asthma model by using polyclonal antibody targeted at acidic mammalian chitinase or chitinase inhibitor allosamidin (Zhu et al., 2004). Other studies showed that IL-13 given intratracheally elevated Ym1 and Ym2 levels in BAL fluid from mice in vivo (Webb et al., 2001). Although Ym1 and Ym2 do not contain chitinolytic activity as the two conserved acidic residues (Asp and Glu) required for chitinase activity are missing in the catalytic domain, they have been shown to specifically bind to carbohydrate moieties such as GlcN oligomers and other glycosaminoglycans such as heparin and heparan sulfate to regulate eosinophil chemotaxis, inflammation and tissue remodeling (Owahasi et al., 2000; Chang et al., 2001). In this study, we observed that MK-571 significantly blocked the expression of acidic mammalian chitinase, Ym1, and Ym2 proteins in bronchoalveolar lavage fluid, and the mRNA levels of acidic mammalian chitinase, Ym1, and Ym2 in lung tissues from ovalbumin-sensitized and ovalbumin-challenged mice. As acidic mammalian chitinase,

Ym1 and Ym2 have been implicated in Th2-mediated immune responses, the down-regulation of chitinase expression by MK-571 is a novel anti-inflammatory action of leukotriene CysLT₁ receptor antagonist in asthma. Down-regulation of chitinases by MK-571 may be linked to the inhibition of pulmonary IL-13 expression as shown in the gene expression study.

Consistent with previous reports from our laboratory and others (Kasper et al., 2002; Koopmans et al., 2004; Schaub et al., 2004; Zhao et al., 2005), allergic patients and mice with allergic airway inflammation produced excessive amount of surfactant protein-D. Surfactant protein-D is a member of the collagenous calcium-dependent lectin (collectin) family, and is synthesized and secreted by airway epithelial cells (Hackzku et al., 2004). The up-regulation of surfactant protein-D production is likely a self-protective response to allergic airway inflammation as surfactant protein-D has been shown to lower blood eosinophilia, pulmonary inflammatory cell infiltration, allergen-specific IgE level and Th2 cytokine levels (Madan et al., 2001). MK-571 markedly suppressed bronchoalveolar lavage fluid level of surfactant protein-D protein and lung tissue surfactant protein-D mRNA. It has been shown that IL-13 is a potent stimulator of surfactant protein-D production from airway epithelial cells (Badour et al., 2002). The mechanism by which MK-571 could down-regulate surfactant protein-D level in asthma may be associated with the repression of IL-13 production.

Lungkine is a member of the glutamic acid-leucine-arginine (ELR)-CXC chemokine family. It has been shown to widely distribute in mouse bronchoepithelial cells and regulate neutrophil migration in mouse airways (Rossi et al., 1999; Chen et al., 2001). Consistent with our previous finding (Zhao et al., 2005), lungkine expression was increased in mouse airway following ovalbumin challenge. MK-571 inhibited the up-regulation of lungkine protein and mRNA levels in ovalbumin-sensitized and ovalbumin-challenged mouse lungs. In our preliminary studies, neither LTD4 nor MK-571 was able to alter the mRNA expression of lungkine in the airway epithelial cell line LA4 (data not shown). Therefore, the ability of MK-571 to suppress the lavage level of lungkine in mouse asthma model is likely due to leukotriene CysLT₁ receptor antagonism on other cell types such as inflammatory cells that can regulate the functions of bronchoepithelium. Nevertheless, human orthologue of lungkine has not been identified and, therefore, its role in human asthma remains to be established.

Actins exist in a variety of isoforms and their distributions are tissue specific. They may perform distinct functions in cell structure and motility (Herman, 1993; Khaitlina, 2001). Unlike α -actin, which is mainly expressed in muscle cells and functions as part of the muscle contraction machinery, γ -actin is ubiquitously expressed and plays important role in cell structural support (Khaitlina, 2001). Our study showed that γ -actin was up-regulated in lavage fluid of mouse asthma model, which was reversed by treatment with leukotriene CysLT₁ receptor antagonist. Reverse transcription-polymerase chain reaction study showed no change in mRNA levels in lung tissue under all treatment conditions (data not shown). The relationship between γ -actin and asthma requires further investigations.

In conclusion, leukotriene CysLT₁ receptor antagonist MK-571 elicited complex pharmacological effects on protein expression in allergic airway inflammation in mice. In particular, a family of chitinases and lungkine, two putative pro-inflammatory proteins, has been identified for the first time to be suppressed by leukotriene CysLT₁ receptor antagonism in allergic airway inflammation. Down-regulation of acidic mammalian chitinase and lungkine can be considered as novel anti-inflammatory actions of leukotriene CysLT₁ receptor antagonist in asthma.

Acknowledgements

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