Pantethine Down-Regulates Leukocyte Recruitment and Inflammatory Parameters in a Mouse Model of Allergic Airway Inflammation

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Background:
Migration of leukocytes into airways is the hallmark of allergic asthma. The aim of this study was to target the pathological process using pantethine, a pleiotropic natural compound which has been recently shown to down-regulate chemokine-driven T cell migration.

Material/Methods:
Mice were sensitized to the Leishmania LACK antigen, then treated or not treated with pantethine and exposed to LACK or saline aerosol. After sacrifice of the animals, cells in the bronchoalveolar lavage were analyzed and inflammatory parameters were determined to evaluate inflammation seriousness.

Results:
As compared to untreated animals, pantethine-treated animals displayed a moderated response to the allergen, as documented by decreased infiltration of inflammatory cells (all types), in addition to reduced levels of lung Th2 cytokines and circulating LACK-specific IgE.

Conclusions:
These data reveal the potential therapeutic importance of pantethine to moderate allergic asthma pathology. The compound has been previously shown to exert a broad range of protective activity in animals and in humans, with few or no adverse effects.

MeSH Keywords:
Anti-Allergic Agents • Asthma • Cell Migration Inhibition • Mice • Pantetheine

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Background

Allergic asthma is a chronic inflammatory lung disease characterized by airway hyper-responsiveness, lung eosinophilia, and airway remodeling [1]. The upstream pathological event is the infiltration of T lymphocytes into airways, followed by recruitment of eosinophils, leading to Th2 cytokine production, with ultimate secretion of allergen-specific IgE by B lymphocytes, as well as mastocyte activation and release of histamine and pro-inflammatory factors. The recruitment of inflammatory cells is under the control of the chemokine/chemokine receptor CXCL12/CXCR4 axis, which therefore plays a pivotal role in the development of lung inflammation [2]; drugs that alter the chemokine/receptor complex block experimental allergic asthma [3].

In vitro experiments allowed us to show that the natural compound pantethine impairs CXCL12-driven mouse or human T lymphocytes chemotaxis and transendothelial migration [4], in connection with its known hypolipidemic properties [5] and its stabilizing effect on cell membrane lipids [6]. Pantethine consists of 2 pantetheine residues linked by a disulfide bond (Figure 1A). In the intestinal tract, the –SS– group is reduced, yielding pantethine, which is phosphorylated by pantothenate kinase (PanK; 2.7.1.33) [7] to generate 4’-phosphopantetheine (4’-PP). The latter forms the active moiety of coenzyme A (CoA), an essential cofactor of lipid synthesis and degradation. The aim of this study was to investigate in vivo impediment of T cell migration by pantethine, using a mouse model of allergic airway inflammation. We determined airway invasion by inflammatory cells as well as the immunological response to the allergen, namely inflammatory cytokines and allergen-specific antibodies known to be related with severity of airways diseases [8].

Material and Methods

Mice and induction of allergic airway inflammation

Female BALB/cAnN mice were purchased from Janvier, France. All animals were raised under specific pathogen-free conditions at the animal facility of IPMC (University of Nice) and used at 6 weeks of age. At days 0 and 7, all the animals were submitted to a sensitization procedure which consisted of 2 i.p. injections of 10 μg of LACK protein precipitated in 2 mg of aluminum hydroxide (alum) [9] (Figure 1B). Then, the mice were randomly distributed into 4 groups, each of 6 animals. Starting from day 17, treated animals received 8 daily i.p. injections of 5 or 7.5 mg of pantethine, whereas untreated animals received a saline solution. Then, starting at day 19, treated and shame-treated mice were exposed to a LACK (1 mg/ml) aerosol challenge for 30 min on 5 consecutive days. Control animals were injected and challenged with saline. Aerosolization was performed using a Passport aerosol compressor (Invacare Corporation, Elyria, OH) connected to a 6500 cm³ box that served as the deposition chamber for the mice. The animals were anesthetized and sacrificed on day 25. The effect of the treatment was evaluated using the cellular content of the bronchoalveolar lavage (BAL) as well as the levels of lung cytokines and circulating LACK-specific IgE and IgG1. Experiments were conducted following protocols approved by the DNAX Animal Care and Use Committee.

Analysis of bronchoalveolar lavage cells

After mice were sacrificed and blood samples were collected, a canula was placed into the trachea, and the lung was washed 4 times with 1 ml of pyrogen-free saline warmed to 37°C. For differential cell count, cells were stained with monoclonal antibodies to CCR3 (R&D Systems), Gr1, CD3, and CD19 (Becton Dickinson) and analyzed using a FACSCalibur flow cytometer equipped with CellQuest software. Eosinophils were defined as CCR3+CD3–CD19–, neutrophils as Gr-1hiCD3–CD19–, and lymphocytes as CD3+CD19+.

Figure 1. Mouse sensitization and pantethine treatment. (A) The pantethine molecule with its constituents; vitamin B5 is pantothenic acid. (B) Schematic view of the protocol used. Mice were sensitized with 2 i.p. injections of LACK protein in alum. The animals (6 per group) then received 8 daily i.p. injections of pantethine (5 or 7.5 mg) or saline and they were challenged with LACK aerosol. The control group was injected and challenged with saline.
Determination of circulating LACK-specific IgE and IgG1 levels

For IgE, plates coated with anti-IgE antibody were incubated with serum dilutions and then biotinylated LACK antigen was added. For IgG1, plates coated with the LACK protein were incubated with serum dilutions and then biotinylated anti-IgG1 antibody was added. In both cases, horseradish peroxidase-conjugated streptavidin and its substrate tetramethylbenzidine (TMB) were used for detection. The 450 nm OD was determined by spectrometry.

Cytokine assays

Lung tissue was homogenized on ice using a tissue-tearer (Biospec Products, Racine, WI) in 350 μl of extraction buffer (HEPES 50 mM, NaCl 0.5 M, NP40 0.2%, and protease inhibitor). Samples were centrifuged at 13 000 rpm for 10 min at 4°C. Supernatants were analyzed for the determination of IL-4, IL-5, IL-10, and IL-13 levels using the Becton Dickinson Multiplex Immunoassays system.

Statistical analysis

Following Wang [10], statistical analyses were performed using ANOVA with SPSS. Data are expressed as means ± standard deviations (SD) or represent individual values (n=6 animals per group); * p<0.05, ** p<0.01 or *** p<0.001 indicated statistical significance.

Results

Effect of pantethine treatment on lung leukocyte recruitment

The number of cells in the bronchoalveolar lavage (BAL) was 1.2×10⁶ in LACK-challenged mice, in contrast to 1.8×10⁵ in saline-challenged ones (p<0.0001) (Figure 2A). Following pantethine treatment, BAL count was reduced by more than 60% (p<0.007). Eosinophils, a major cellular effector of the pathology, formed the main body of infiltrating leukocytes in LACK-challenged mice, whereas they were absent in PBS-challenged ones. Pantethine treatment drastically reduced their number by more than 70% (p<0.009) (Figure 2B).

Effect on circulating LACK-specific IgE and IgG1

LACK-specific IgE levels were almost undetectable in the serum of saline-challenged animals, while they were high in LACK-challenged ones. The levels were reduced by 64% in pantethine-treated mice compared to untreated ones (p=0.001) (Figure 2A). Following pantethine treatment, BAL count was reduced by more than 60% (p<0.007). Eosinophils, a major cellular effector of the pathology, formed the main body of infiltrating leukocytes in LACK-challenged mice, whereas they were absent in PBS-challenged ones. Pantethine treatment drastically reduced their number by more than 70% (p<0.009) (Figure 2B).

Effect on lung cytokines

LACK-challenged mice displayed significantly higher lung levels of IL-4, IL-5, IL-10, and IL-13 as compared to saline-challenged
Figure 3. Lung levels of LACK-specific IgE and IgG1. Each dot represents a single mouse and the bar represents the mean value of each experimental group (n=6 per group). Significant difference of the mean between treated and untreated animals, * p<0.05; *** p<0.001.

Figure 4. Lung cytokine levels. Each dot represents a single mouse and the bars represent the mean value of each experimental group (n=6 per group). Significant difference of the mean between treated and untreated animals, * p<0.05; ** p<0.01; *** p<0.001.
animals (Figure 4). Pantethine treatment markedly reduced the levels of the pro-inflammatory cytokines IL-4 (p=0.0008) and IL-13 (p=0.0082). The treatment was effective also for IL-5 and IL-10, but only at the highest pantethine dose (p=0.041 and p=0.0071, respectively).

**Discussion**

We explored at the potential protective effect of the low-molecular-weight thiol pantethine against allergic airway inflammation, based on the report that the compound inhibits chemokine-induced migration of T cells [4], a central pathophysiological process in allergic asthma [11–13].

We found that airway infiltration by inflammatory cells was significantly reduced in pantethine-treated mice as compared to saline-treated mice. The event was not limited to lymphocytes, as it involved all inflammatory cell types, including eosinophils, which are assumed to play a broad role in disease development [14]. Accordingly, levels of factors that play a critical role in the disease [15,16] were significantly reduced, as shown by lung levels of Th2 cytokines such as IL-4, IL-5, and IL-13, as well as circulating levels of LACK-specific IgE and IgG1. The protective effect of inhibiting the IgE allergic pathway has been demonstrated in patients with severe persistent asthma, using humanized mAb to IgE [17].

It should be emphasized that pantethine treatment was applied after the LACK injections, meaning that it was efficient in already-sensitized animals. The compound has already been shown to target complex cell pathological pathways, such as the cell response to pro-inflammatory factors associated with cerebral malaria, resulting in the prevention of the cerebral syndrome [6]. It also attenuated the brain energy crisis linked to Parkinson’s disease, with subsequent protection against dopaminergic injury [18,19].

**Conclusions**

Our work shows that pantethine is a potential protective drug against allergic asthma. Among the products known to attenuate the disease, pantethine has the advantage of being a well-tolerated natural compound. It has been already used in humans against various pathologies, with no detectable adverse effects [20]. Based on a previous work showing that it drastically attenuates the diffusion of cancer metastases [21], the present report confirms that pantethine inhibits pathogenic cell migration, meaning that it could provide a distinct appropriate therapy for allergic asthma.

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