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Intranasal azelastine and mometasone exhibit a synergistic effect on a murine model of allergic rhinitis☆

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ABSTRACT

Purpose: The purpose of this study was to compare the anti-allergic effects of the combination of azelastine and mometasone with those of either agent alone in a *Dermatophagoides farinae* (Derf)-induced murine model of allergic rhinitis (AR).

Materials and methods: Forty BALB/c mice were divided into five groups: azelastine (A), mometasone (M), a combination of azelastine and mometasone (MA), Derf, and control. Derf served as the allergen. Allergic symptom scores, eosinophil counts, and serum Derf-specific IgE levels were measured. The mucosal levels of mRNAs encoding interferon (IFN)-γ, T-bet, interleukin (IL)-4, GATA-3, Foxp3, IL-17, and ROR-γt were determined by real-time polymerase chain reaction. The T-bet, GATA-3, Foxp3, and ROR-γt results were confirmed by Western blotting.

Results: Nose-rubbing motions; the levels of mRNAs encoding IL-4, GATA-3, and ROR-γt; and tissue eosinophil count were reduced in the MA compared with those in the Derf group (all P values <0.05). The levels of mRNAs encoding GATA3 and IL-4 mRNA [synthesized by T helper (Th)2 cells] were reduced and that of mRNA encoding Foxp3 was increased in the MA compared with those in the Derf and A groups. Western blotting confirmed these findings.

Conclusion: We found that the combination of intranasal azelastine and mometasone synergistically suppressed Th17 responses and (reciprocally) elevated Treg responses. Therefore, this combination not only ameliorated allergic inflammation by suppressing Th2 responses, but also usefully modified the Treg/Th17 balance.

1. Introduction

Allergic rhinitis (AR) affects >500 million people worldwide [1,2]. It is the fifth most common chronic disease [3] and the most common chronic disease among children [4] in the United States. The direct cost of AR has been estimated to be approximately ~$7 billion annually in the United States [2], and indirect costs may bring that figure to ~$9.7 billion [5]. The cost is estimated at €4260 per patient per year in Europe [6]. Comorbid diseases such as asthma and atopy further increase AR-related treatment costs [7,8].

The principal current guideline-based therapies for AR are medical in nature, including corticosteroids and antihistamines (via the intranasal or oral route) or oral leukotriene receptor antagonists [9]. Intranasal corticosteroids are as effective as oral corticosteroids, and the intranasal route is preferred because there are fewer side effects [1,9]. Intranasal antihistamine therapy has recently become popular, being more efficacious than oral therapy [10]. The onset of action is rapid [11] and the efficacy comparable to that of oral corticosteroids in terms of symptom control [11–13].

However, AR treatment is challenging; many patients have moderate-to-severe disease and do not respond adequately to treatment [14]. Up to 74.4% of all patients use multiple treatments in attempts to achieve symptom relief [8,14–17], although the evidence that such approaches are effective is limited [2]. Physicians tend to prescribe combination treatments featuring oral antihistamines and intranasal corticosteroids [16,18], although evidence of their efficacy is lacking [19–22].

Many studies have tested medical combinations for the treatment of AR. However, none of intranasal steroid-plus-oral antihistamine, oral antihistamine-plus-leukotriene receptor antagonist, or intranasal steroid-plus-leukotriene receptor antagonist treatment has afforded any pronounced clinical benefit [9]. Although an intranasal steroid-plus-intranasal oxymetazoline regimen afforded some relief, rebound concerns indicate that intranasal oxymetazoline should be limited to a few days [9]. However, combinations of intranasal steroids and antihistamines have been suggested to be more effective than monotherapies when

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treating AR [23–26]. In addition, the safety and tolerability profiles of the combinations were favorable [27]. These supposedly superior results have not yet been investigated at the immunological level. Thus, in the present study, we compared the effects of a combination of intranasal steroid and antihistamine with those of either agent alone in a murine model of AR.

2. Materials and methods

2.1. Experimental animals

Six-week-old healthy female BALB/c mice (20–30 g) were used. All experiments were performed with the approval of the Institutional Animal Care and Use Committee of the Catholic University of Korea.

2.2. Reagents

Dermatophagoides farinae (Derf) crude body extract (Arthropods of Medical Importance Resource Bank, College of Medicine, Yonsei University, Seoul, South Korea) was dissolved in water prior to use. Mice were assigned to receive azelastine (Bukwang Pharm., Co., Ltd., Seoul, South Korea), mometasone (Hanmi Pharm., Co., Ltd., Seoul, South Korea), or Motesone® (mometasone furoate 50 mcg, Azelastine HCl 140 mcg; Hanmi Pharm., Co., Ltd.).

2.3. The AR model and the treatment protocol

Forty mice were randomized into five groups: control ($n = 8$), Derf (AR, $n = 8$), M (mometasone administered after challenge, $n = 8$), A (azelastine administered after challenge, $n = 8$), and MA (Motesone® administered after challenge, $n = 8$). Allergen sensitization and challenge in the context of the murine AR model are summarized in Fig. 1. Briefly, on days 0, 7, and 14, all mice except those in the control group were immunized via intraperitoneal injection of 100 μg of Derf and 1 mg of aluminum hydroxide (Thermo Scientific, Waltham, MA, USA). After 1 week, all sensitized mice were intranasally challenged with 20 μg of Derf daily for 6 consecutive days. Mice in the M group received intranasal mometasone (0.2 μg) on days 21, 22, 23, 24, 25, and 26. Mice in the A group received intranasal azelastine (0.14 μg) on days 21, 22, 23, 24, 25, and 26. Mice in the MA group received intranasal Motesone® (0.58 μg; 0.56 μg of azelastine and 0.2 μg of mometasone) on days 21, 22, 23, 24, 25, and 26. The control group received intranasal PBS.

2.4. Allergic symptoms induced after allergen challenge

The numbers of sneezes and nose-rubbing motions during 15-min periods after final allergen challenge were recorded and compared among experimental groups by blinded observers.

2.5. Derf-specific immunoglobulin E (IgE) levels in serum

Serum Derf-specific IgE levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Indoor Biotechnologies, Cardiff, UK).

2.6. Real-time polymerase chain reaction (PCR)

Nasal mucosae were removed and real-time PCR was used to quantitate the levels of mRNAs encoding interferon (IFN)-γ, T-bet, interleukin (IL)-4, GATA-3, Foxp3, IL-17, and ROR-γt. Total RNA was extracted from nasal mucosa using TRIzol reagent (Invitrogen, Waltham, MA, USA); the first strands were reverse-transcribed using random primers (Takara, Otsu, Japan). The oligonucleotide primer sequences were as follows: IFN-γ forward, 5′-AGAGCCAGATTATCTCTTTCTACCTCAG-3′ and IFN-γ reverse, 5′-CCTTTTTCGCCTTGCTGTTG -3′; T-bet forward, 5′-GCCAGGGAACCGCTTATA-3′ and T-bet reverse, 5′-CCTTGTTGTTGGTGAGCTTTA-3′; IL-4 forward, 5′-TCAACCCCCAGCTAGTTGTC-3′ and IL-4 reverse, 5′-AAATATGCGAAGCACCTTGG-3′; GATA-3 forward, 5′-CTGGATGGCGGCAAAGC-3′ and GATA-3 reverse, 5′-GTGGGCGGGAAGGTGAA-3′; Foxp3 forward, 5′-GAAAGCGGATACCAAATGA-3′ and Foxp3 reverse, 5′-CTGTGAGGACTACCGAGCC-3′; ROR-γt forward, 5′-ACCTCCACTGCCAGCTGTGTGCTGTC-3′ and ROR-γt reverse, 5′-TCATTTCTGCACTTCTGCATGTAGACTGTCCC-3′; IL-17 forward,5′-TTTAACTCCCTTGGCGCAAAA-3′ and IL-17 reverse, 5′-CTTTCCCTCCGCATTGACAC-3′; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5′-GCACAGTCAAGGCCGAGAAT-3′ and GAPDH reverse, 5′-GCCTTCTCCATGGTGGTGAA-3′. The levels of mRNAs encoding IFN-γ, T-bet, IL-4, GATA-3, Foxp3, IL-17, ROR-γt, and GAPDH were determined by real-time PCR using the CFX96 Real Time PCR platform (Bio-Rad, Hercules, CA, USA) and an iQ SYBR Green Supermix kit (Bio-Rad). All results were normalized to GAPDH expression and are shown as fold increases over control expression levels.

2.7. Western blotting

Western blots were used to quantify the relative expression levels of T-bet, GATA-3, Foxp3, and ROR-γt in nasal mucosae from the control, Derf, M, A, and MA groups. We calculated grayscale ratios between the expression levels of target genes and GAPDH; these indicate the relative expression levels of target genes. The antibodies used were the anti-IFN-γ antibody sc-59,992, the anti-T-bet antibody sc-21,003, the anti-IL-4 antibody sc-1260, and the anti-GATA-3 antibody sc-9009 (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.8. Statistical analysis

All data are expressed as means ± standard deviations (SDs). Among-group differences were analyzed using the Kruskal-Wallis test.

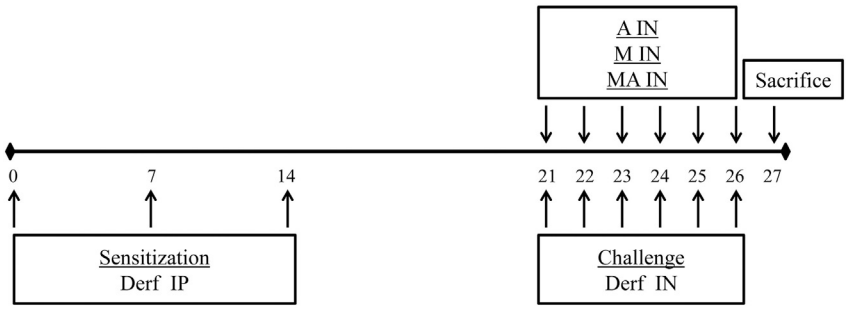

Fig. 1. Schematic representation of our experimental allergic rhinitis model and treatment protocol. A, azelastine; M, mometasone; MA, Motesone® (mometasone furoate 50 mcg/dose, azelastine HCl 140 mcg/dose); Derf, *Dermatophagoides farinae*; IP, intraperitoneal administration; IN, intranasal administration. The time units are days.

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