International Immunopharmacology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Genomic and non-genomic effects of glucocorticoids on allergic rhinitis model in mice

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ARTICLE INFO

Article history:
Received 3 October 2012
Received in revised form 22 March 2013
Accepted 27 March 2013
Available online xxxx

Keywords:
Glucocorticoid
Mometasone furoate
Genomic effect
Non-genomic effect
Sneezing
Nasal rubbing

ABSTRACT

Glucocorticoids (GCs) are well known for their anti-inflammatory effects, which are elicited through a transcriptional mechanism via a cytosolic glucocorticoid receptor (cGR)-mediated genomic effect. However, recent *in vitro* studies report that GCs can act as a membrane glucocorticoid receptor (mGR). This study aimed to examine whether mometasone furoate (MF) influences the nasal symptoms induced by histamine, substance P, ATP. Furthermore, the influences of various compounds on MF action were studied *in vivo*. The mice were intranasally administered with nasal symptom-inciting agents, and the occurrences of sneezing and nasal rubbing were counted. MF repressed the nasal symptoms caused when it was administered 10, 30 and 60 min before the induction of nasal symptoms. The repressive effect observed 10 min after the administration of MF was inhibited by RU486, a GR antagonist, but not by actinomycin D, a transcriptional inhibitor. In contrast, the repressive effect observed 60 min after the administration of MF was inhibited by RU486 and actinomycin D. Therefore, the effects observed 10 and 60 min after the MF administration were classified as non-genomic and genomic effects, respectively. The non-genomic effect suppressed the nasal symptoms induced by m-3M3FBS, a phospholipase C (PLC) activator, and was inhibited by U-73122, a PLC inhibitor. The genomic effect was inhibited by N-(p-amylcinnamoyl) anthranilic acid, a phospholipase A_2 (PLA_2) inhibitor. These results indicate that MF has a non-genomic effect through repression of the activation of PLC via the mGR, and MF has also a genomic effect that was influenced by the inhibition of PLA_2 through transcriptional regulation via cGR.

1. Introduction

Allergic rhinitis is one of the most common immune-mediated diseases, and its main symptoms are sneezing, pruritus, rhinorrhea and nasal obstruction. The pathogenesis of the allergic reaction initially involves the interaction of allergens with a specific immunoglobulin (IgE) antibody bound to the surface of mast cells and basophils on the nasal mucosa. As a result, many symptoms associated with allergic rhinitis are caused by the release of mediators, including histamine, substance P, leukotrienes and cytokines from mast cells and eosinophils [1].

In clinical practice, glucocorticoids (GCs) are used to treat allergic rhinitis [2,3]. GCs are part of the normal feedback mechanism in the immune system that reduces inflammation. Therefore, they are used in medicine to treat diseases that are caused by an overactive immune system, such as allergies, asthma, autoimmune diseases, and sepsis [4]. GCs are a class of steroid hormone that binds to the cytosolic glucocorticoid receptor (cGR), which is a member of the nuclear receptor superfamily and is present in almost every vertebrate animal cell [5,6]. The activated GR complex in turn upregulates the expression of anti-inflammatory proteins in the nucleus and represses the expression of pro-inflammatory proteins [7–9]. Because of these genomic action mechanisms, a few hours are required after the administration of the GCs for their effects to become apparent [10,11]. In clinical practice, however, the inhibitory effect of topical administration of GCs on nasal symptoms appears in a few minutes after administration. For example, Tillmann et al. [12] reported that nasal itching was markedly reduced 10 min after the administration of dexamethasone. It has thus become increasingly apparent that GRs are also present on the cell membranes (membrane GR [mGR]) and that GCs can exert non-genomic effects via the mGR [13–15].

Genomic effects involve the regulation of the transcription of specific target genes, ultimately resulting in the production of proteins that positively or negatively modulate cell function [9,16,17]. In contrast to genomic effects, which tend to be slow in onset and delayed in recovery, non-genomic effects occur within minutes, are reversible immediately after the removal of the GCs, and are insensitive to substances that block RNA transcription [18,19]. Therefore, we propose that GCs have some effects in addition to those mediated by transcriptional regulation. Non-genomic effects of GCs have been demonstrated *in vitro*, but are uncertain *in vivo*. The present study was

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1567-5769/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.intimp.2013.03.030

therefore undertaken to clarify the genomic and non-genomic effects of GCs on nasal symptoms using a mouse allergic rhinitis model.

2. Materials and methods

2.1. Animals

Five week-old female ICR mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in an air-conditioned room with controlled temperature (24 ± 2 °C) and humidity (55 ± 15%). Food and water were given *ad libitum*. Ten animals were used for the investigation of the nasal symptoms. All the procedures involving the animals were conducted in accordance with the guidelines for animal experiments at Okayama University Advanced Science Research Center, and all the procedures were licensed by the Animal Research Control Committee of Okayama University.

2.2. Materials

The following reagents were obtained from the sources shown in the parentheses: histamine dihydrochloride (Sigma, St. Louis, MO, USA) and substance P (Peptide Institute, Inc., Osaka, Japan). These reagents were dissolved in saline. Adenosine 5′-triphosphate disodium salt hydrate (ATP, Sigma) was dissolved in phosphate-buffered saline (PBS). RU486 (Cayman Chemicals, Ann Arbor, MI, USA) and U-73122 (Tocris, Bristol, UK) were suspended in 10% dimethyl sulfoxide (DMSO)-saline. Actinomycin D (Wako Pure Chemicals, Osaka, Japan) was suspended in 10% ethanol-saline. m-3M3FBS (Sigma) was suspended in 10% DMSO 10% Tween80-saline. N-(p-amylcinnamoyl) anthranilic acid (ACA, Sigma) was suspended in 20% DMSO-PBS. A mometasone furoate (MF, MSD, Tokyo, Japan) nasal spray (500 μg/mL) was used as the model GCs. The mice were given a nasal instillation of the all drugs in volume of 2 μL into the bilateral cavities using a micropipette.

2.3. Evaluation of the nasal symptoms in mice

Before the experiment, the animals were placed in an observation cage (31 × 25 × 18 cm) for approximately 10 min for acclimatization. After the nasal instillation of 2 μL of the drugs into the bilateral cavities using a micropipette, the animals were placed back into the observation cage (1 animal per cage), and the frequencies of sneezing and nasal rubbing in 15 min were counted.

2.4. Statistical analysis

All the values are expressed as the mean ± SEM. The statistical evaluation of the results was performed by one-way ANOVA, followed by the Dunnett's test or, when only two means were to be compared, the unpaired Student's *t*-test. A probability value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of MF on the nasal symptoms induced by histamine in mice

Fig. 1A shows the incidence of sneezing and nasal rubbing over the 15 min period after the intranasal administration of histamine. Histamine at a dose of 5 μmol/site significantly elicited nasal symptoms compared with the saline treatment. MF at a dose of 1 μg/site was administered 5, 10, 30, 60 or 120 min before the induction of nasal symptoms by histamine (5 μmol/site). As shown in Fig. 1B, MF inhibited the sneezing and the nasal rubbing induced by histamine. Significant effects of MF were observed when it was administered 10, 30 and 60 min before the induction of nasal symptoms by histamine compared with the saline treatment.

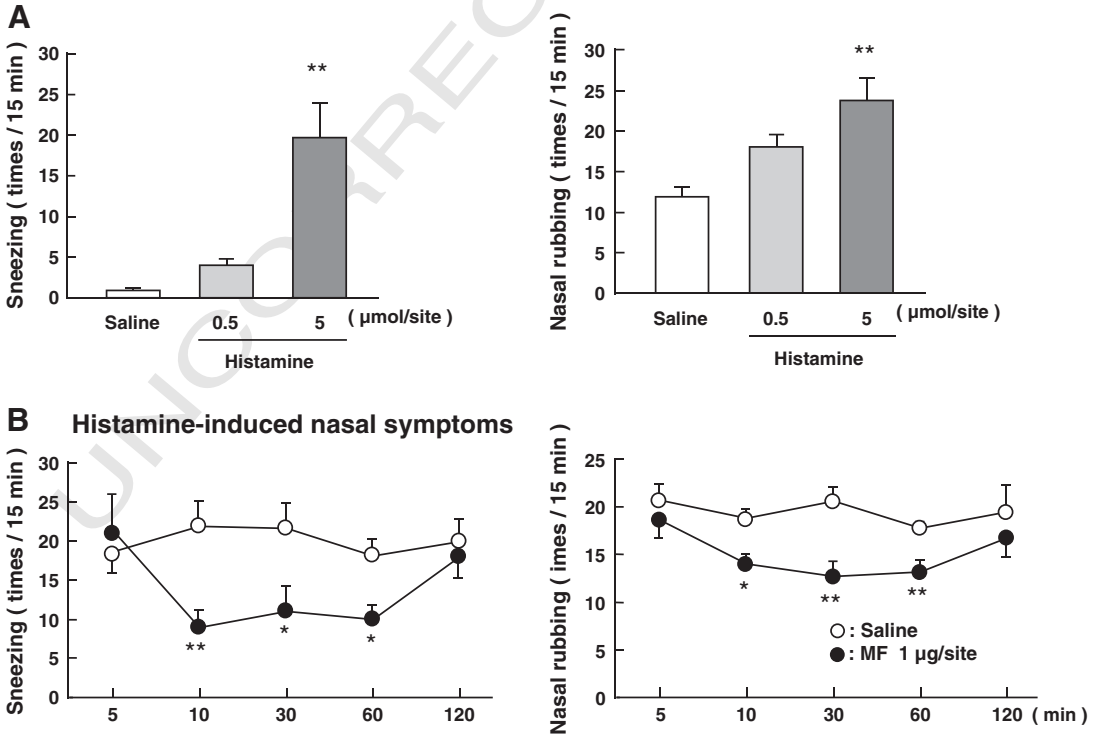

Fig. 1. (A) Sneezing and nasal rubbing induced by an intranasally administration of histamine in mice. The mice were given an intranasal administration of saline or histamine (0.5 and 5 μg/site) and the sneezing and nasal rubbing were counted for 15 min. Each column and vertical bar represents the mean ± SEM (n = 10). **: Significantly different from saline-treated group at $p < 0.01$. (B) Effects of MF on sneezing and nasal rubbing induced by histamine. The mice were given an intranasal administration of saline or MF (1 μg/site) 5, 10, 30, 60 or 120 min before the induction of nasal symptoms. Each value represents the mean ± SEM (n = 10). *, **: Significantly different from saline-treated group at $p < 0.05$ and $p < 0.01$, respectively.

Please cite this article as: Kusaka E, et al, Genomic and non-genomic effects of glucocorticoids on allergic rhinitis model in mice, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.03.030