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Disodium cromoglycate inhibits asthma-like features induced by sphingosine-1-phosphate

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ABSTRACT

Compelling evidence suggests the involvement of sphingosine-1-phosphate (S1P) in the pathogenesis of asthma. The systemic administration of S1P causes asthma like features in the mouse involving mast cells. In this study we investigated whether disodium cromoglycate (DSCG), administered as a preventative treatment as in human therapy, could affect S1P effects on airways. BALB/c mice, treated with DSCG, received subcutaneous administration of S1P. Bronchi and pulmonary tissues were collected and functional, molecular and cellular studies were performed. DSCG inhibited S1P-induced airway hyperreactivity as well as pulmonary inflammation. DSCG decreased the recruitment of solely mast cells and B cells in the lung. IgE serum levels, prostaglandin D_2, mucus production and IL-13 were also reduced when mice were pretreated with DSCG. S1P induced pulmonary expression of CD23 on T and B cells, that was reversed by DSCG. Conversely, S1P failed to upregulate CD23 in mast cell-deficient Kit $^{W-sh/W-sh}$ mice. In conclusion we have shown that DSCG inhibits S1P-induced asthma like features in the mouse. This beneficial effect is due to a regulatory action on mast cell activity, and in turn to an inhibition of IgE-dependent T and B cells responses.

1. Introduction

Mast cells are widely known for their harmful activity during lung allergic inflammation [1,2]. Their infiltration into airway smooth muscle cell layer characterizes allergic asthma associated with airway hyper-reactivity [3,4]. The ability of mast cells to produce a variety of bioactive products makes these cells the first-line regulators in many immune functions [5,6].

Sphingosine-1-phosphate (S1P) has been recognised as a new inflammatory mediator secreted by activated mast cells and involved in both innate and adaptive immunity [7,8]. Cross-linking of IgE for its high affinity receptor (FcεRI) on mast cells activates sphingosine kinases (SPKs), which triggers phosphorylation of sphingosine to generate S1P. This event plays a relevant role in cell degranulation, leading to release of allergic pro-asthma mediators [9,10]. In addition, S1P can be released by mast cells to amplify their response through binding S1P receptors in a paracrine and autocrine manner [11,12].

Compelling evidence suggests an involvement of SPK/S1P pathway in the pathogenesis of chronic asthma [13]. Elevated levels of S1P in bronchoalveolar lavage (BAL) fluid were recovered from allergic asthma patients after ragweed antigen challenge [14]. In support, administration of S1P exacerbates antigen-induced airway inflammation in mice [15]. Furthermore, the treatment of ovalbumin (OVA)-sensitised mice with an inhibitor of SPKs ameliorates the development of bronchial smooth muscle hyper-responsiveness in a mast cell dependent manner [16,17]. Accordingly we published data demonstrating that the systemic administration of S1P induces several asthma-like features in the

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mouse involving mast cells [17–20]. Recently Oskeritzian et al. demonstrated that pretreatment with anti-S1P mAb inhibits mast cell activation in vitro as well as development of airway inflammation and mast cell activation in vivo [21].

Mast cell-derived lipid mediators and their receptors represent interesting therapeutic targets for treating allergic inflammation. However, multiple functions and complex biology are associated with these mediators. Disodium cromoglycate (DSCG) belongs to chromones that still have a niche role in the treatment of asthma and allergies [22]. DSCG, when inhaled prior to challenge, is capable to inhibit both the early and late phase response to a variety of inhaled allergens, bronchial constrictor agents and exercise [23]. The DSCG-like drugs are known as 'mast cell stabilizers' due to the fact that they prevent the release of mast cell histamine upon stimulation by different agonists [24]. However, many other actions of DSCG-like drugs have been also reported [25–29].

In this study we investigated whether DSCG could affect disease manifestation in a murine asthma model induced by S1P.

2. Materials and methods

2.1. Animals

Female BALB/c and mast cell-deficient Kit $^{W-sh/W-sh}$ [30,31] mice (8weeks) were purchased from Charles River Laboratories (Milan, Italy). The animals were housed in a controlled environment and provided with standard rodent chow and water. All mice were housed with a 12 h light dark cycle and were allowed food and water ad libitum. All animals were allowed to acclimate for four days prior to experiments. The experiments described have been carried out in accordance with Italian regulations on protection of animals used for experimental and other scientific purpose (Ministerial Decree 116/92) as well as with the European Economic Community regulations (Official Journal of E.C. L 358/1 12/18/1986). The animal studies were approved by the local ethical committee of the University of Naples Federico II on 20/07/2012 (approval number 2012/0081821).

2.1.1. Animal injections and harvest

BALB/c mice received s.c. injection of S1P (10 ng, equivalent to 0.5 μg/Kg; Enzo Life Science, Italy) in sterile saline containing BSA (0,001%) on days 0 and 7 [18,20]. Part of these mice received disodium cromoglycate (DSCG; i.p. 50 mg/Kg; Sigma Aldrich, Italy) 30 min prior to S1P administration. Vehicle mice receiving 0.1 ml of sterile saline containing BSA (0,001%) were used as control. On day 14 and 21 mice were anesthetised with i.p. ketamine/xylazine and euthanized by bleeding. Bronchial reactivity and lung function were assessed at 21 days. In another set of experiments mice were sacrificed at 14 days to take bronchoalveolar lavage and pulmonary tissues used for functional and molecular studies. Each lung was divided into two parts. One part was frozen in liquid nitrogen for 2 h before storage at −86 °C for cytokine measurements, and the other was fixed in 10% neutralized buffered formalin for histopathological and immunohistochemical detection. In another set of experiments mast cell-deficient Kit $^{W-sh/W-sh}$ mice received s.c. injection of S1P (0.5 μg/Kg; Enzo Life Science, Italy) in sterile saline containing BSA (0,001%) on days 0 and 7. Mice were sacrificed at 14 days and lungs fixed in 10% neutralized buffered formalin for immunohistochemical analysis. Each experimental group consisted of 6 mice.

2.1.2. Depletion of mast cells

BALB/c mice were pretreated i.p. every 12 h for four days with the mast cell degranulator compound 48/80 (CM48/80) dissolved in PBS (phosphate-buffered saline) and injected at 200 μl/cavity according to the following schemes: day 1, 0.6 mg/kg; day 2, 1.0 mg/kg; day 3, 1.2 mg/kg; day 4, 2.4 mg/kg. The administration of S1P was performed 24 h after the last dose of compound 48/80 [32,33].

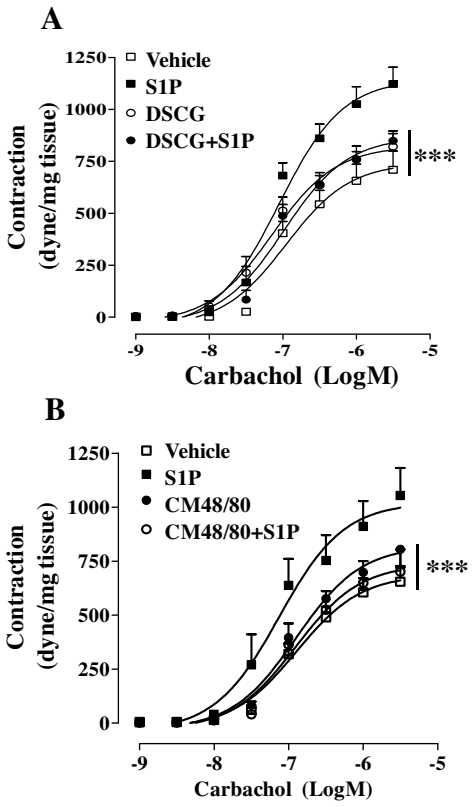

Fig. 1. DSCG inhibits S1P-induced bronchial hyper-responsiveness.
BALB/c mice received subcutaneous administration of S1P (10 ng) or vehicle (BSA 0.001% in phosphate-buffered saline) on days 0 and 7. A: DSCG (50 mg/kg) was administered i.p. 30 min before S1P or vehicle on days 0 and day 7. On day 21 bronchial reactivity to carbachol was assessed (***$P < 0.001$ vs. S1P). B: The mast cell degranulator compound 48/80, dissolved in phosphate-buffered saline, was injected at 200 μl/cavity to mice i.p. every 12 h for four days, according to the following schemes: day 1, 0.6 mg/kg; day 2, 1.0 mg/kg; day 3, 1.2 mg/kg; day 4, 2. 4 mg/kg. The administration of S1P was performed 24 h after the last dose of compound 48/80. On day 21, bronchial reactivity to carbachol was assessed (***$P < 0.001$ vs. S1P). Data represents means ± SEM; $n = 6$ mice per group.

2.2. Airway reactivity

2.2.1. Bronchial reactivity

BALB/c mice were sacrificed and bronchial tissues were rapidly dissected and cleaned from fat and connective tissue. Rings of 1–2 mm length were cut and placed in organ baths mounted to isometric force transducers (type 7006, Ugo Basile, Comerio, Italy) and connected to a Powerlab 800 (AD Instruments). Rings were initially stretched until a resting tension of 0.5 g was reached and allowed to equilibrate for at least 30 min. In each experiment, bronchial rings were challenged with carbachol (10^{-6} mol/L) until the response was reproducible. Once a reproducible response was achieved, the bronchial reactivity was assessed performing a cumulative concentration-response curve to carbachol (1×10^{-8}–3×10^{-5} mol/L).

2.2.2. Isolated perfused mouse lung preparation

Lung function was assessed using an isolated and perfused mouse lung model [34]. Lungs were perfused in a non-recirculating fashion through the pulmonary artery at constant flow of 1 ml/min resulting in a pulmonary artery pressure of 2–3 cm H_2O. The perfusion medium used was RPMI 1640 lacking phenol red (37° C). The lungs were ventilated by negative pressure (−3 and −9 cm