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Short communication

Adenine suppresses IgE-mediated mast cell activation

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ABSTRACT

Nucleobase adenine is produced by dividing human lymphoblasts mainly from polyamine synthesis and inhibits immunological functions of lymphocytes. We investigated the anti-allergic effect of adenine on IgE-mediated mast cell activation in vitro and passive cutaneous anaphylaxis (PCA) in mice. Intraperitoneal injection of adenine to IgE-sensitized mice attenuated IgE-mediated PCA reaction in a dose dependent manner, resulting in a median effective concentration of 4.21 mg/kg. In mast cell cultures, only adenine among cytosine, adenine, adenosine, ADP and ATP dose-dependently suppressed FcεRI (a high affinity receptor for IgE)-mediated degranulation with a median inhibitory concentration of 1.6 mM. It also blocked the production of LTB_4, an inflammatory lipid mediator, and inflammatory cytokines TNF-α and IL-4. In addition, adenine blocked thapsigargin-induced degranulation which is FcεRI-independent but shares FcεRI-dependent signaling events. Adenine inhibited the phosphorylation of signaling molecules important to FcεRI-mediated allergic reactions such as Syk, PLCγ2, Gab2, Akt, and mitogen activated protein kinases ERK and JNK. From this result, we report for the first time that adenine inhibits PCA in mice and allergic reaction by inhibiting FcεRI-mediated signaling events in mast cells. Therefore, adenine may be useful for the treatment of mast cell-mediated allergic diseases. Also, the upregulation of adenine production may provide another mechanism for suppressing mast cell activity especially at inflammatory sites.

1. Introduction

Adenine is an ancestor molecule (Miller and Urey, 1959) and a component of biomolecules, such as nucleic acids and ATP. It can be produced from adenosine and 5′-methylthioadenosine (MTA) by purine nucleoside phosphorylase and MTA-phosphorylase, respectively (Avila et al., 2004). MTA is formed from decarboxylated *S*-adenosylmethionine in the synthesis of spermidine and spermine during cell proliferation and is metabolized to yield 5-methylthioribose-1-phosphate and adenine by MTA-phosphorylase. Therefore, adenine production from MTA degradation contributes 85–97% of total adenine synthesis in dividing human lymphoblastoid cells (Kamatani and Carson, 1981).

Several biologic functions of adenine have been reported previously. Adenine inhibits human lymphoblast growth (Hershfield et al., 1977; Snyder et al., 1978), mitogen-stimulated transformation, cytoplasmic immunoglobulin production, and natural killer activity of human mononuclear leukocytes (Ito and Uchino, 1976; Kishi et al., 1985). In contrast to its growth inhibitory effects, adenine has protective role in survival of rat Purkinje cells (Watanabe et al., 2003) and erythrocytes in whole blood storage (Simon et al., 1962). It causes a biphasic effect on cAMP level in rat peritoneal mast cells (Sullivan et al., 1975a) and potentiates 48/80 induced degranulation (Sullivan et al., 1975b). Recently, adenine specific G protein-coupled receptor has been cloned and characterized in a mouse brain (Bender et al., 2002; von Kugelgen et al., 2008) and a pig kidney where it inhibits Na^{+}-ATPase activity (Wengert et al., 2007).

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Mast cells are associated with various allergic diseases (Bischoff, 2007; Gregory and Brown, 2006). Mast cells express FcεRI, the high affinity receptor for IgE (Gilfillan and Tkaczyk, 2006). Administration of multivalent antigen induces cross-linking of FcεRIs that are pre-occupied with IgEs specific to antigen, initiating signaling events resulting allergic reaction. The activated mast cells release intracellular granules that store preformed inflammatory mediators such as histamine, TNF-α and proteases by the degranulation process. Also, they synthesize and secrete inflammatory lipids and cytokines TNF-α and IL-4. A Src-family tyrosine kinase (SFK), Lyn, is associated with FcεRI (Kihara and Siraganian, 1994) and is activated in response to cross-linking of FcεRI caused by antigen treatment. Lyn phosphorylates tyrosine residues on immunoreceptor tyrosine-based activation motif (ITAM) in beta and gamma chain subunits of FcεRI complex. Phosphorylated ITAM of the gamma chain recruits Syk. This binding increases enzymatic activity of Syk. It phosphorylates tyrosine residues on the adaptor proteins linker of activated T cell (LAT) and Grb2-associated binder 2(Gab2) to provide docking sites for signaling molecules to ensure the phosphorylation and activation of phospholipase Cγ and the mobilization of calcium to generate critical signals for degranulation (Siraganian et al., 2010). Also activation of phosphatidylinositol 3-kinase and mitogen-activated protein (MAP) kinases promotes transcription factors for the production of cytokines and synthesis of eicosanoids, inflammatory lipids.

In this study we describe the anti-allergic effect of adenine and its action mechanism in mast cells. We show that adenine suppresses IgE-mediated passive cutaneous anaphylaxis (PCA) in mice and allergic reactions in mast cells such as FcεRI-mediated degranulation and secretion of an inflammatory lipid and cytokines by inhibiting FcεRI-mediated signaling events.

2. Materials and methods

2.1. Materials

The following materials were purchased from the indicated commercial sources: adenine, dinitrophenol (DNP)-specific monoclonal IgE, DNP-HSA, Evans blue dye, ADP, ATP, cytosine and thapsigargin from Sigma–Aldrich; PP2 from Calbiochem; fetal bovine serum from Gibco/Life Technology; Eagle's minimal essential medium and trypsin from Lonza; recombinant IL-3 from Peprotech; phospho-tyrosine mouse mAb (P-Tyr-100), anti-phospho-Syk (Tyr 525/526), anti-phospho-Src (Tyr 416), anti-Akt, anti-phospho-Akt (Ser473 and T308), anti-ERK and anti-phospho-ERK (Thr202/Tyr204), anti-phospho-p38 MAPK (Thr180/Tyr182) and anti-p38 MAPK from Cell Signaling Technology; anti-phospho-JNK (Thr183/Tyr185) and anti-JNK from Invitrogen; BD Calcium Assay Kit and ELISA sets for mouse TNF-α and IL-4 cytokine from BD Biosciences; TRIzol RNA extraction reagent from Invitrogen; Reverse Transcription Master Premix (5) from ELPIS Biotech; LTB_4 ELISA kit from Enzo Life sciences; ECL chemiluminescence kit from Millipore; RBL-2H3 cell line from American Type Culture Collection.

2.2. Induction of passive cutaneous anaphylaxis in mice

The BALB/c mice were purchased from DBL Korea and kept under specific pathogen-free conditions and maintained according to the guidelines of the Institutional Animal Care and Use Committee of Chungnam National University. PCA was performed as described with some modifications (Kim et al., 1999). Briefly, the mice were anaesthetized and injected intradermally with DNP-specific IgE (0.5 μg each mouse) into the ear. After 24 h, DNP-HSA antigen (250 μg) in 200 μl PBS containing 1% Evans blue was injected intravenously. PBS, adenine (2, 5 and 10 mg/kg) or ketotifen (10 mg/kg) was administered by intra-peritoneal injection 1 h before antigen treatment. The mice were sacrificed 1 h after antigen challenge, followed by the removal of ears for measurement of the amount of dye extravasated. The dye was extracted overnight from the ears in 500 μl of formamide at 63 °C. The absorbance was measured in a microplate reader at 620 nm.

2.3. Preparation and culture of bone marrow-derived mast cells and RBL-2H3 cells

For the preparation of bone marrow-derived mast cells (BMMCs), femurs of 6 weeks old BALB/c mouse were taken and bone marrow was flushed with PBS and collected. The cells were suspended and cultured in RPMI 1640 medium containing 10% heat inactivated FBS, 1% penicillin and streptomycin, 2 mM glutamine, 50 μM 2-mercaptoethanol, 25 mM HEPES [pH 7.4] supplemented with 10 ng/ml IL-3 in 5% CO_2 incubator at 37 °C. The non-adherent cells were reseeded to the fresh medium twice a week. By 4–5 weeks in culture, the cells were used for the further experiments after verifying that cells have granules stained with toluidine blue. RBL-2H3 cells were cultured in complete media (EMEM supplemented with 15% (v/v) FBS, 100 U/ml of penicillin and 100 μg/ml of streptomycin) at 37 °C in 5% CO_2 incubator.

2.4. Sensitization and stimulation of BMMCs and RBL-2H3 mast cells

RBL-2H3 cells and BMMCs were sensitized with 100 ng/ml DNP-specific IgEs overnight in a complete growth medium. The cells were stimulated with 100 ng/ml DNP-HSA antigen after washing and preincubation for 30 min with Tyrode's buffer (10 mM HEPES [pH 7.4], 125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 5.6 mM glucose) to measure degranulation, calcium ion mobilization, and LTB_4 secretion and to do immunoblots of signaling molecules. To measure cytokine mRNAs, cytokine secretion and cell viabilities, a complete growth medium was used to incubate the cells.

2.5. Measurement of degranulation in BMMCs and RBL-2H3 mast cells

The activity of a granule marker enzyme, β-hexosaminidase, in the medium and inside the cells after antigen stimulation was determined by a colorimetric assay (Ozawa et al., 1993) with a few modifications. Briefly, cells were transferred to 24-well cluster plates (1×10^5 cells per 0.4 ml per well) and sensitized with IgE overnight. The cultures were washed twice and replaced with Tyrode's buffer (0.2 ml per well) for RBL-2H3 cells and BMMCs. The cultures were incubated for 30 min with adenine, adenosine, ADP, ATP, or cytosine before stimulation with antigen for 20 min. For thapsigargin-induced degranulation, RBL-2H3 cells prepared like above were stimulated with thapsigargin (5 μM) for 20 min instead of antigen treatment. Production of *p*-nitrophenol from 5 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide was measured by a colorimetric assay. The values were expressed as percentages of β-hexosaminidase released into the medium.

2.6. Measurement of cytokine mRNA levels

RBL-2H3 cells were transferred to 6-well cluster plates (2×10^5 cells per 2 ml per well), sensitized and stimulated as described in Section 2.4. After preincubation with adenine, cells were stimulated with antigen for 0.5 h or 2 h. The cells were washed with cold PBS. RNAs were extracted with TRIzol solution according to the manufacturer's protocol and 1 μg of total RNA was used to synthesize