Glucocorticosteroids Rescue Basophils from Dasatinib-Augmented Immunoglobulin E-Mediated Histamine Release

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**Abstract**

**Background:** Dasatinib is a multikinase inhibitor active against several tyrosine kinases including ABL, KIT, Lyn and Btk. Apart from its known antileukemic activity, the drug produces several side effects including edemas and pleural effusions, which are supposedly triggered by activated immune cells. Effusion formation can be treated effectively by glucocorticosteroids. We have recently shown that low concentrations of dasatinib (<0.1 \( \mu \text{M} \)) promote IgE-dependent secretion of histamine in basophils, especially in allergic individuals. In the current study, we asked whether glucocorticosteroids inhibit dasatinib-induced activation of basophils.

**Methods:** Basophils were preincubated with dexamethasone, prednisolone and hydrocortisone for 24 h, and then exposed to an anti-IgE antibody (normal basophils) or the allergens Bet v 1 and Phl p 5 (allergic patients) with or without low concentrations of dasatinib (0.025 \( \mu \text{M} \)). After incubation, basophils were examined for histamine release and expression of CD63 and CD203c.

**Results:** All three glucocorticosteroids were found to counteract IgE-dependent and dasatinib-enhanced histamine release in basophils in nonallergic and allergic individuals. In addition, glucocorticosteroids were found to inhibit anti-IgE-induced upregulation of CD63 and CD203c in the presence or absence of dasatinib. The inhibitory effects of glucocorticosteroids were dose-dependent (effective range: 1–10 \( \mu \text{M} \)) and seen in all donors examined.

**Conclusions:** Glucocorticosteroids rescue IgE receptor cross-linked basophils from additional co-stimulatory effects of low-dose dasatinib which may have clinical implications in dasatinib-treated patients.

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**Key Words**

Basophils · Dasatinib · Glucocorticosteroids · Histamine · Immunoglobulin E receptor

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**Introduction**

Dasatinib is a novel small molecule-type drug that inhibits a number of tyrosine kinases including PDGFR, KIT, BCR/ABL, Lyn, and Btk [1–4]. Based on its strong effect on BCR/ABL, dasatinib has been used to treat patients with imatinib-resistant chronic myeloid leukemia (CML) [5]. Moreover, dasatinib inhibits the growth of...
neoplastic mast cells harboring imatinib-resistant mutants of KIT [6–8]. However, a number of clinical trials have shown that dasatinib also produces several side effects, including cytopenia and pleural as well as pericardial effusions [5, 9–11]. Pleural effusions are especially seen quite frequently in dasatinib-treated patients with CML [10, 11], but are not seen in patients treated with other BCR/ABL kinase inhibitors. Although various kinase and nonkinase targets of dasatinib [12, 13] have been implicated in dasatinib-related effusion formation, the exact mechanisms underlying this drug side effect remain at present unknown. The observation that glucocorticosteroids can counteract the formation of pleural effusions in dasatinib-treated patients [9–11, 14] suggests that the activation of immune cells may be a pathogenetic factor. We have recently shown that low concentrations of dasatinib (<0.1 μM) promote IgE-mediated secretion of histamine from human basophils, especially in allergic individuals, whereas higher doses of dasatinib even block histamine secretion [15]. We examined the effects of three glucocorticosteroids on basophils exposed to allergen or anti-IgE antibody and low concentrations of dasatinib. The results of our study show that glucocorticosteroids effectively counteract low-dose dasatinib-induced enhancement of IgE-mediated histamine release in human basophils.

Methods

Monoclonal Antibodies and Other Reagents

The anti-IgE monoclonal antibody (mAb) E124.2.8 (Dr.2), the FITC-labeled mAb CLB-gran12 (CD63), and the PE-conjugated mAb 97A6 (CD203c) were purchased from Immunotech (Mar- seille, France). Dasatinib was kindly provided by Dr. F.Y. Lee (Bristol-Myers Squibb, New Brunswick, N.J., USA). The tyrosine kinase inhibitors (TKIs) imatinib and nilotinib were kindly provided by Dr. E. Buchdunger and Dr. P.W. Manley (Novartis Pharma AG, Basel, Switzerland). INNO-406 was purchased from Selleck Chemicals (Riverside, Calif., USA). Stock solutions of TKIs and glucocorticosteroids were prepared by dissolving in dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany). Reombinant interleukin-3 (IL-3) was from Novartis, dexamethasone, prednisolone and hydrocortisone from Sigma-Aldrich (St. Louis, Mo., USA), and RPMI 1640 medium and fetal calf serum from PAA Laboratories (Pasching, Austria). The reombinant allergens rBet v 1 and rPhl p 5 were obtained from Biomay (Vienna, Austria).

Enrichment of Human Blood Basophils

Peripheral blood was obtained from 12 healthy individuals and 8 patients allergic to Bet v 1 and/or Phl p 5 [16]. Informed consent was obtained in each case. The study was approved by the institutional review board (Medical University of Vienna) and conducted in accordance with the declaration of Helsinki. Peripheral blood was collected in heparin-containing tubes. Basophils were enriched by dextran sedimentation (histamine release experiments) or were recovered together with mononuclear cells (MNCs) after centrifugation over Ficoll (surface staining experiments). The percentage of basophils in dextran preparations ranged from 0.1 to 1.5%, and the percentage of basophils in MNC preparations ranged from 0.3 to 2%. Cell viability was always >90% as assessed by the trypan blue exclusion test.

Histamine Release Assay

The histamine release assay was performed on dextran-enriched basophils (healthy donors, n = 10; allergic donors, n = 8) essentially as described [15, 17]. Before challenged with TKIs and anti-IgE, cells were preincubated in control medium or in medium containing dexamethasone, prednisolone or hydrocortisone (0.001–10 μM) at 37°C for 24 h following published protocols [18, 19]. After incubation, cells (3 × 10^6/ml) were incubated in medium in the presence or absence of TKIs (dasatinib, imatinib, nilotinib, INNO-406, each 0.025 or 1 μM) for 30 min at 37°C, washed and thereafter incubated with various concentrations of anti-IgE antibody E124.2.8 (0.001–10 μg/ml) or allergen (0.001–1 μg/ml) in histamine release buffer at 37°C for another 30 min. Cells were then centrifuged at 4°C, and the cell-free supernatants and total suspensions recovered and analyzed for histamine content by radioimmunoassay (RIA, Immunotech). Histamine release was calculated as the percent of total (cellular + extracellular) histamine. In a separate set of experiments, various concentrations of TKIs (0.001–1 μM) were applied to establish dose-response relationships. In these experiments, cells were exposed to one concentration of anti-IgE (1 μg/ml) or allergen (0.1 μg/ml) to induce histamine liberation. Histamine release was expressed as a percentage of total histamine. All experiments were performed in triplicate.

Antibody Staining and Flow Cytometry

To examine the effects of anti-IgE, allergens or drugs on the expression of activation-linked cell surface antigens on basophils, flow cytometry experiments were performed. MNCs were preincubated with RPMI 1640 medium plus 0.5 ng/ml IL-3 in the absence or presence of glucocorticosteroids (dexamethasone, hydrocortisone and prednisolone, each 10 μM) at 37°C for 24 h. Loss of viability (and apoptosis) of basophils during incubation with glucocorticosteroids was excluded by morphology, trypan blue exclusion and staining for active caspase 3 (not shown). Cells were incubated with dasatinib (0.001–1 μM) for 15 min at 37°C, washed and then incubated with anti-IgE mAb E124.2.8 (1 μg/ml) at 37°C for 15 min. Cells were then washed in phosphate-buffered saline supplemented with EDTA (20 mM) and stained with the PE-labeled CD203c mAb 97A6 and the FITC-labeled CD63 mAb CLB-gran12 as reported [15]. The expression of cell surface antigens was quantified by multicolor flow cytometry on a FACScan or FACSCalibur (Becton Dickinson Biosciences, San Jose, Calif., USA) [15, 20]. Basophils were identified as CD203c-positive cells. Anti-IgE-induced upregulation of CD63 or CD203c on basophils was calculated from mean fluorescence intensities (MFI) obtained with stimulated (MFIstim) and unstimulated (MFIcontrol) cells, and was expressed as a stimulation index (SI) calculated as MFIstim : MFIcontrol [20].

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Statistical Evaluation of Data

To determine the significance of differences in histamine secretion and surface antigen expression in basophils after preincubation with glucocorticosteroids, dasatinib, anti-IgE or control medium, standard statistical tests including Student’s t test were applied. Results were considered significantly different when the p value was <0.05.

Results

Low Concentrations of Dasatinib Promote IgE-Dependent Histamine Release in Human Blood Basophils

Confirming previous results [15], low concentrations of dasatinib were found to promote allergen-induced histamine release and anti-IgE-induced histamine release from human blood basophils (fig. 1a). By contrast, at higher concentrations (>0.1 μM), dasatinib suppressed IgE-dependent secretion of histamine (fig. 1a). The other TKIs tested, namely imatinib, nilotinib and INNO-406, did not promote IgE-mediated histamine secretion in basophils at low or high concentrations (fig. 1b–d). Neither dasatinib nor the other TKIs tested (0.001–1.0 μM) were found to induce histamine release in basophils in the absence of anti-IgE (not shown).

Glucocorticosteroids Counteract Dasatinib-Induced Enhancement of IgE-Mediated Histamine Release in Blood Basophils

A number of previous studies have shown that glucocorticosteroids inhibit IgE-dependent secretion of histamine in human basophils [18, 19]. In the present study, we were able to confirm these observations using dexamethasone, hydrocortisone and prednisolone, as well as basophils obtained from normal donors or patients allergic to Bet v 1 or Phl p 5 (fig. 2). In addition, we were able to show that all 3 glucocorticosteroids tested also counteract histamine secretion from basophils preincubated with low concentrations of dasatinib (0.025 μM) and then triggered with anti-IgE or allergen (fig. 2a, b). Glucocorticosteroid effects on histamine secretion in anti-IgE- or allergen-exposed basophils and in dasatinib-preincubated basophils challenged with anti-IgE or allergen were dose dependent (fig. 3a) and observed at all dasatinib concentrations tested (fig. 3b). The solvent control (DMSO, 1:1,000) did not modulate histamine release in basophils (not shown).
Glucocorticosteroids Counte ract IgE-Dependent Upregulation of the Expression of CD63 and CD203c in Blood Basophils in the Presence or Absence of Dasatinib

CD63 and CD203c are well established activation antigens expressed on basophils. Notably, exposure to anti-IgE or allergen is followed by an increased expression of CD63 and CD203c [20–22]. In this study, we confirmed that low concentrations of dasatinib enhanced the anti-IgE- or allergen-induced upregulation of the expression of CD63 and CD203c on basophils (fig. 4). In addition, we were able to show that dexamethasone, hydrocortisone and prednisolone inhibit the anti-IgE-induced or allergen-induced upregulation of CD63 and CD203c on basophils in the presence or absence of low concentrations of dasatinib (0.025 μM; fig. 4). However, even at high concentrations, the effects of the glucocorticosteroids on the upregulation of CD63 and CD203c were less pronounced when compared to the effects on IgE-dependent histamine release.

Discussion

Dasatinib is a multikinase inhibitor used to treat patients with imatinib-resistant or intolerant CML [1, 5]. At high concentrations, this BCR/ABL kinase blocker exhibits anti-inflammatory and immunosuppressive effects. Drug side effects include edema formation and pleural as well as pericardial effusions, which are specific for this...
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BRC/ABL kinase inhibitor [5, 9, 11]. In most patients, effusion formation can be kept under control using diuretics and short-term glucocorticosteroids [5, 9, 10]. The exact mechanism of action of glucocorticosteroids in these patients remains unknown. We have recently shown that dasatinib, at low concentrations, can promote IgE-dependent secretion of histamine in basophils [15]. We here show that glucocorticosteroids counteract activation and histamine release in IgE receptor cross-linked basophils even when cells were exposed to low concentrations of dasatinib.

A number of previous studies have shown that glucocorticosteroids inhibit IgE-dependent histamine release in human basophils [18, 19]. Since it has been described that basophils need to be incubated with glucocorticosteroids for 24 h to suppress histamine release [18, 19], thereby contrasting the rapid suppression of interleukin-4 release [23], we selected a 24-hour incubation period in our experiments. In these experiments, we were able to confirm this drug effect and show that even in basophils exposed to low concentrations of dasatinib, glucocorticosteroids can counteract IgE-dependent histamine release. In most allergic donors, glucocorticosteroids exhibited a strong effect with almost complete inhibition of histamine secretion. However, in a few donors, glucocorticosteroids were unable to completely block histamine secretion in basophils exposed to anti-IgE or allergens in the presence of low concentrations of dasatinib. These data suggest that dasatinib-mediated activation of blood basophils may sometimes involve glucocorticosteroid-independent mechanisms and targets.

A number of previous studies suggest that IgE receptor cross-linking on basophils is accompanied by upregulation of various cell surface antigens, including CD63 and CD203c [15, 20–22]. However, to date little is known about mechanisms underlying the upregulation of such activation antigens following IgE receptor cross-linking, or about the effects of various anti-inflammatory drugs. We have recently shown that high concentrations of dasatinib (1 μM) block IgE-dependent upregulation of CD63 and CD203c [15]. In the present study, we have shown that low concentrations of dasatinib promote IgE-dependent upregulation of CD63 and CD203c on basophils. In addition, we were able to show that the three glucocorticosteroids applied counteract the upregulation of CD63, and, less effectively, the upregulation of CD203c on IgE receptor cross-linked basophils in the absence or presence of low concentrations of dasatinib. An interest-

Fig. 3. Dose-dependent effects of dasatinib and dexamethasone on basophil histamine release. a Dextran-enriched normal basophils were preincubated in control medium or in medium containing various concentrations of dexamethasone for 24 h. Thereafter, cells were incubated in the absence or presence of dasatinib (0.025 μM) at 37°C for 30 min, and were then exposed to anti-IgE (1 μg/ml) in histamine release buffer for 30 min. Total and released histamine was quantified by RIA. Histamine release is expressed as the percentage of total histamine and represents the mean ± SD of triplicates in one representative experiment.

b Dextran-enriched basophils from 3 healthy donors were preincubated in control medium or in medium containing dexamethasone (1 μM) for 24 h. Thereafter, cells were incubated with or without various concentrations of dasatinib (as indicated) for 30 min. Then, cells were incubated with anti-IgE (1 μg/ml) in histamine release buffer for another 30 min. Released histamine was expressed as the percentage of histamine released after anti-IgE stimulation (control + anti-IgE). Results show the mean ± SD of 3 donors. *p < 0.05 compared to the control (at the same concentration of dasatinib).
The mechanism and biochemical basis of dasatinib-induced enhancement of histamine release in IgE receptor cross-linked basophils remain at present unknown. One possibility may be that some of the dasatinib targets counteract IgE-dependent reactions in basophils. One candidate for an inhibitory signaling molecule might be Lyn, a signal transduction molecule that binds to dasatinib [15] and has been implicated as a dual regulator of secretory responses in basophils and mast cells [25, 26].
Notably, depending on the cell type and culture condition, Lyn has been discussed as an enhancer or blocker of histamine release [25, 26]. In addition, it has been described that other Src inhibitors, such as PP1 and PP2, also enhance IgE-mediated histamine secretion in basophils in a small window of concentrations lower than those that inhibited IgE-dependent histamine release from basophils [27].

To test the hypothesis that Lyn may be a relevant target responsible for the dasatinib-induced upregulation of histamine release in basophils, we applied a second Lyn inhibitor, INNO-406 [28]. However, under the experimental conditions applied, INNO-406 did not promote histamine secretion and the same was found with the other TKIs tested. These data suggest that other dasatinib targets apart from Lyn are responsible for the enhancement of histamine secretion and the same was found with the other Dasatinib levels in biological fluids are below 0.1 nM for most of the daytime in these patients.

In summary, our data show that glucocorticosteroids effectively counteract basophil activation provoked by IgE receptor cross-linking in the presence of dasatinib. This observation may have clinical implications and may explain why glucocorticosteroids are beneficial in patients who develop pleural effusions during dasatinib treatment.

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References


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