Glucocorticosteroids rescue basophils from dasatinib-augmented IgE-mediated histamine release

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Running Head: Corticoids rescue basophils from dasatinib-effects

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Abstract

Background: Dasatinib is a multi-kinase inhibitor active against several tyrosine kinases including ABL, KIT, Lyn, and Btk. Apart from its known anti-leukemic 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 µM) 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 hours, and were 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 µM). 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 non-allergic 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 µM) 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.
Introduction

Dasatinib is a novel small molecule type drug that inhibits a number of tyrosine kinases (TK) 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 harbouring 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]. Especially pleural effusions are 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 non-kinase 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 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 (mAb) and other reagents

The anti-IgE mAb E124.2.8 (Dε2), the FITC-labeled mAb CLB-gran12 (CD63), and the PE-conjugated mAb 97A6 (CD203c) were purchased from Immunotech (Marseille, France). Dasatinib was kindly provided by Dr. F.Y. Lee (Bristol-Myers Squibb, New Brunswick, NJ). The TK 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, CA). Stock solutions of TKIs and glucocorticosteroids were prepared by dissolving in dimethyl-sulfoxide (DMSO) (Merck, Darmstadt, Germany). Recombinant interleukin-3 (IL-3) was from Novartis, dexamethasone, prednisolone, and hydrocortisone from Sigma-Aldrich (St. Louis, MO), and RPMI 1640 medium and fetal calf serum (FCS) from PAA laboratories (Pasching, Austria). The recombinant 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 (MNC) 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 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 challenge 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 hours following published protocols [18,19]. After incubation, cells (3 x 10⁶/ml) were incubated in medium in the presence or absence of TKIs (dasatinib, imatinib, nilotinib, INNO-406, each 0.025 µM or 1 µM) for 30 minutes 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 minutes. 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 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 percent of total histamine. All experiments were performed in triplicates.
Antibody staining and flow cytometry

To examine the effects of anti-IgE, allergens, or drugs on expression of activation-linked cell surface antigens on basophils, flow cytometry experiments were performed. MNC were preincubated with RPMI 1640 medium plus 0.5 ng/ml IL-3 in the absence or presence of glucocorticosteroids (dexamethasone, hydrocortisone, prednisolone, each 10 µM) at 37°C for 24 hours. 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 then incubated with dasatinib (0.001-1 µM) for 15 minutes at 37°C, washed, and then incubated with anti-IgE mAb E124.2.8 (1 µg/ml) at 37°C for 15 minutes. Cells were then washed in phosphate-buffered saline (PBS) supplemented with EDTA (20 mM), and then stained with the PE-labeled CD203c mAb 97A6 and the FITC-labeled CD63 mAb CLB-gran12 as reported [15]. Expression of cell surface antigens was quantified by multicolor flow cytometry on a FACScan or FACSCalibur (Becton Dickinson Biosciences, San Jose, CA) [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 (MFI_{stim}) and unstimulated (MFI_{control}) cells, and was expressed as stimulation index = SI (MFI_{stim}/MFI_{control}) [20].

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 the 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-1d). 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-2b). 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, 1:1000) did not modulate histamine release in basophils (not shown).

**Glucocorticosteroids counteract IgE-dependent upregulation of 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 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 upregulation of CD63 and CD203c were less pronounced when compared to effects on IgE-dependent histamine release.
Discussion

Dasatinib is a multi-kinase-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 BCR/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 hours 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 allergen 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, so far little is known about mechanisms underlying
upregulation of such activation antigens following IgE receptor cross-linking, and
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 show 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
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 interesting observation was that higher concentrations of
glucocorticosteroids were required to suppress anti-IgE-induced or anti-IgE plus
dasatinib-induced upregulation of CD63 on basophils when compared to
glucocorticoid concentrations required to suppress histamine release. This observation
points to the fact, that IgE receptor-dependent histamine release and IgE receptor-
dependent upregulation of CD63 are based on two different mechanisms, which
confirms recently published results [24].

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 signalling 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 release. Notably, dasatinib has been described to bind to a large number of kinase and non-kinase targets in CML cells and basophils [12,13,15].

The pathogenesis of effusion-formation, especially pleural effusions, in patients receiving dasatinib remains uncertain. The frequency of effusion formation may be lower when the drug is administered at 100 mg once daily compared to 70 mg twice daily [29] although still effusion formation may occur [30]. Other studies have suggested that activated immune cells contribute to effusion formation occurring in dasatinib-treated patients [31]. The exact type of immune cells and mechanisms involved remain unknown. An attractive hypothesis would be that basophils and mast cells contribute to effusion formation through the release of histamine and other
vasoactive mediators, especially when cells are (pre)activated by an allergen or other stimuli. Indeed, several of these patients may develop pleural effusions during or shortly after an inflammatory event (e.g. respiratory infection) or allergic event. Whereas diuretics alone show only limited effects on pleural effusions in these patients, it has been described that administration of glucocorticosteroids usually lead to a rapid improvement in most of these patients [10,14]. Another question is whether such low concentrations of dasatinib that caused upregulation of IgE-dependent mediator release, are expected to occur in vivo. Since dasatinib has a very short half life (less than 3 hours) in vivo [32], one can indeed expect, that 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 act beneficial in patients who develop pleural effusions during dasatinib treatment.
Authorship

Authors’ Contribution: HH, KB, VW, and MK performed key laboratory experiments, KM and RV provided vital reagents and recruited allergic donors, and PV contributed the study design and approved the final version of the manuscript.

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30. Krauth MT, Herndlhofer S, Schmook MT, Mitterbauer-Hohendanner G, Schlägl E,
Valent P: Extensive pleural and pericardial effusion in chronic myeloid leukemia during treatment with dasatinib at 100 mg or 50 mg daily. Haematologica 2011;96:163-166.


Figure Legends

Fig. 1. Effects of tyrosine kinase inhibitors on IgE-dependent histamine release in basophils.
Dextran-enriched basophils from healthy individuals were exposed to various concentrations of dasatinib (A), imatinib (B), nilotinib (C), and INNO-406 (D) at 37°C for 30 minutes. Then, cells were stimulated with anti-IgE (1 µg/ml) in histamine release buffer for another 30 minutes. Histamine was quantified in cell lysates and supernatants by RIA. Histamine release is expressed as percentage of total histamine. Results represent the mean±S.D. from triplicates in one representative donor. Almost identical results were obtained in two other donors.

Fig. 2. Glucocorticosteroids inhibit IgE-dependent histamine release in dasatinib-exposed basophils
A: Dextran-enriched basophils obtained from healthy donors were preincubated in control medium with DMSO (1:1000) or medium containing glucocorticoids (DX = dexamethasone, HC = hydrocortisone, PD = prednisolone) for 24 hours. Thereafter, cells were incubated in control medium (open bars) or medium containing dasatinib (0.025 µM; black bars) at 37°C for 30 minutes. Then, cells were incubated with 1 µg/ml anti-IgE. Histamine was quantified in cell lysates and supernatants by RIA. In the left panel, histamine release is expressed as percentage of total histamine and results represent the mean±S.D. from triplicates in one donor. In the right panel, results are expressed as percent of anti-IgE-induced release in each donor (=100%) and
represent the mean±S.D. of seven donors. Asterisk: $p<0.05$. B: Dextran-enriched basophils obtained from patients allergic to Phl p 5 (left panel) or Bet v 1 (middle panel) were preincubated in control medium (with DMSO) or glucocorticoids (DX = dexamethasone, HC = hydrocortisone, PD = prednisolone) for 24 hours. Thereafter, cells were incubated in control medium (open bars) or medium containing dasatinib (0.025 µM; black bars) at 37°C for 30 minutes. Then, cells were incubated with or without recombinant Phl p 5 (0.1 µg/ml) or Bet v 1 (0.1 µg/ml) for 30 minutes. In the left and middle panel, histamine release is expressed as percentage of total histamine and results represent the mean±S.D. from triplicates in 2 representative donors. In the right panel, results are expressed as percent of allergen-induced release in each donor (=100%) and represent the mean±S.D. of three donors. Asterisk: $p<0.05$.

**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 hours. Thereafter, cells were incubated in the absence or presence of dasatinib (0.025 µM) at 37°C for 30 minutes, and were then exposed to anti-IgE (1 µg/ml) in histamine release buffer for 30 minutes. Total and released histamine was quantified by RIA. Histamine release is expressed as percentage of total histamine and represents the mean±S.D. of triplicates in one representative experiment. B: Dextran-enriched basophils from three healthy donors were preincubated in control medium (black bars) or in medium containing dexamethasone 1 µM (open bars) for 24 hours. Thereafter, cells were incubated with or without various concentrations of dasatinib (as indicated) for 30 minutes. Then,
cells were incubated with anti-IgE (1 µg/ml) in histamine release buffer for another 30 minutes. Released histamine was expressed as percent of histamine released after anti-IgE stimulation (Control + anti-IgE). Results show the mean±S.D. of three donors. Asterisk: p<0.05 compared to control (at the same concentration of dasatinib).

**Fig. 4.** Effects of low-dose dasatinib and glucocorticosteroids on IgE-dependent upregulation of expression of CD63 and CD203c on basophils

Mononuclear cells from healthy donors (A,B) and patients allergic to Bet v 1 or/and Phl p 5 (C,D) were preincubated in 0.5 ng/ml IL-3 in control medium with DMSO (Co) or medium containing glucocorticosteroids (DX, dexamethasone; HC, hydrocortisone; PD, prednisolone, 10 µM each) for 24 hours. Then, cells were incubated with or without dasatinib (0.025 µM) at 37°C for 15 minutes, and then were exposed to anti-IgE (A,B) or recombinant allergens (C,D) for 15 minutes. Thereafter, basophils were analyzed for expression of CD63 (left panels) and CD203c (right panels) by flow cytometry. Upregulation of CD63 and CD203c is expressed as stimulation index calculated from the mean fluorescence intensity (MFI) obtained with stimulated cells (MFI_{stim}) and unstimulated cells (MFI_{control}), following the formula: MFI_{stim}:MFI_{control}. A and C show typical results obtained in one individual healthy donor (A) and one representative allergic donor (C). Panels B and D show results obtained in all normal donors (n=3, B) and in all allergic donors (n=5, D). In these analyses (B and D), IgE-dependent plus dasatinib-induced upregulation of CD63 and CD203c is expressed as 100%-stimulation, and results represent the mean±S.D. from all normal donors (n=3) and all allergic donors (n=5) examined. Asterisk: p<0.05 compared to control (IgE-dependent plus dasatinib-induced upregulation=100%).
**Herrmann et al., Figure 1**

**A**

[Graph showing histamine release vs. Dasatinib (µM) with anti-IgE (1 µg/ml).]

**B**

[Graph showing histamine release vs. Imatinib (µM) with anti-IgE (1 µg/ml).]

**C**

[Graph showing histamine release vs. Nilotinib (µM) with anti-IgE (1 µg/ml).]

**D**

[Graph showing histamine release vs. INNO-406 (µM) with anti-IgE (1 µg/ml).]
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