



Immunopharmacology and Inflammation

Anti-allergic effects of nilotinib on mast cell-mediated anaphylaxis like reactions

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ARTICLE INFO

Article history:

Received 23 October 2011

Received in revised form 24 January 2012

Accepted 28 January 2012

Available online 6 February 2012

Keywords:

Nilotinib

Anaphylactic reaction

Mast cell

Histamine

ABSTRACT

Nilotinib is a new orally bioavailable potent tyrosine kinase inhibitor that is used for the treatment of BCR-ABL-positive chronic myelogenous leukemia. However, its effect on mast cell-mediated anaphylactic reaction is still not known. The present study aimed to investigate the effect of nilotinib on the anaphylactic allergic reaction and study its possible mechanism(s) of action. Nilotinib administration prevented systemic anaphylaxis in mice, mediated by compound 48/80, in a dose- and time-dependent manner. Also, nilotinib significantly inhibited ($P < 0.05$) allergic paw edema in rats. Furthermore, nilotinib significantly decreased ($P < 0.05$) the IgE-mediated passive cutaneous anaphylaxis in a dose dependent manner. In addition, nilotinib dose-dependently reduced histamine release from the rat peritoneal mast cells activated either by compound 48/80 or by ovalbumin. Moreover, nilotinib attenuated the secretion of pro-inflammatory cytokine, tumor necrosis factor (TNF)- α expression in the rat peritoneal mast cells. These findings provide evidence that nilotinib inhibits mast cell-derived immediate-type allergic reactions and so it could be a candidate as an anti-allergic agent.

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1. Introduction

Nilotinib is a second-generation tyrosine kinase inhibitor, 30-fold more potent than imatinib, that has been approved for imatinib-resistant BCR-ABL positive chronic myelogenous leukemia (Giles et al., 2010). It inhibits the tyrosine kinase activity of ABL/BCR-ABL, as well as that of the platelet-derived growth factor, discoidin domain and stem cell factor receptors (KIT) (Kantarjian et al., 2006; Manley et al., 2010). Stem cell factor receptors are expressed by haematopoietic progenitor cells, germ cells, and some human tumor cells, but of the mature immune system cells, only mast cells express KIT receptors in significant numbers (Natali et al., 1992). It has been reported that KIT is essential for the growth and survival of human mast cell (Valent, 1994). Thus the inhibition of KIT receptor signaling might prevent mast cell activity.

Mast cells, which are broadly distributed throughout mammalian tissues, play a critical role in a wide variety of biologic responses. Mast cells are the primary effector cell in immediate-type allergic reactions as well as in late reaction like inflammatory responses (Kemp and Lockey, 2002). Activation of mast cell causes the process of degranulation that result in releasing of several mediators (Miyajima et al., 1997). Mast cell degranulation can be elicited by non-immunologic stimulators such as neuropeptides, basic compounds, complement components, and certain drugs. Compound 48/80 and polymers of basic amino acids, such as substance P, are some

of the most potent stimulators of mast cells (Ennis et al., 1980). Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of the mast cell-mediated non-specific anaphylactic reaction (Allansmith et al., 1989). Mast cells can also be activated immunologically by aggregation of their cell surface-specific receptors (Fc ϵ RI) for immunoglobulin E (IgE) by the corresponding antigen. It is known that the anti-IgE antibody induces passive cutaneous anaphylaxis as a typical model for mast cell-dependent immediate type allergic reaction (Alber et al., 1991). Activated mast cells produce histamine as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, and several pro-inflammatory and chemotactic cytokines such as tumor necrosis factor- α (TNF- α) and interleukin (IL)-6, IL-8, IL-4, IL-13 and transforming growth factor- β (TGF- β) (Royer et al., 2001). Modulation of these cytokines secretion from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease.

The present study aimed to investigate the effect of nilotinib on immunologic and nonimmunologic stimulation-mediated anaphylactic reactions. Moreover, the effect of nilotinib on the production of the inflammatory cytokine, TNF- α was studied.

2. Materials and methods

2.1. Drugs and chemicals

Nilotinib was generously supplied by Novartis Pharma AG, Basle, Switzerland. Compound 48/80, disodium cromoglycate (DSCG), ovalbumin (chicken egg, Grade V), Evans blue, gelatin, bovine serum

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albumin (BSA), di-o-phthaldialdehyde, N-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid (HEPES) and metrizamide were purchased from Sigma (St. Louis, MO, USA). Nilotinib was suspended in 0.5% carboxymethylcellulose (CMC) solute. All other chemicals and biochemicals used in this study are of high analytical grade.

2.2. Experimental animals

Male Swiss albino mice (6–8 weeks old, 18–22 g each) and male Wistar rats (7 weeks old, 150–180 g each) were purchased from Urology and Nephrology Center, Mansoura University, Egypt. The animals were housed (6 per cage) in an air-conditioned room maintained at $25 \pm 2^\circ\text{C}$ with regular 12 h light/12 h dark cycle. All procedures involving the animals were conducted in accordance with the protocol approved by the committee on Animal Experimentation of Mansoura University.

2.3. Compound 48/80-induced systemic anaphylaxis

The experiment was carried out according to the method previously described by Jiang et al., 2008. Briefly, mice were given an intraperitoneal injection of 8 mg/kg body weight of the mast cell degranulator, compound 48/80. Nilotinib was administered orally 1 h before the injection of compound 48/80 ($n = 10/\text{group}$) at doses of 5, 10, 50, 100 and 150 mg/kg. Another group was orally administered DSCG (1000 mg/kg) as positive control drug (Shin et al., 2004). Mortality was monitored for 1 h after induction of anaphylactic shock. In the time-dependent experiment, nilotinib (150 mg/kg) was administered 5, 10, 20 and 30 min after compound 48/80 injection ($n = 10/\text{group}$). Mortality was monitored for 1 h after induction of anaphylactic shock.

2.4. Ovalbumin induced paw edema

Paw edema was induced in immunized rats as previously described (Feitosa et al., 2002). Briefly, ovalbumin was dissolved in phosphate-buffered saline (PBS) then mixed with an equal volume of colloidal $\text{Al}(\text{OH})_3$. The rats were sensitized on day 0 by intraperitoneal injections of 30 μg of ovalbumin/rat dispersed in 0.5 ml of PBS and $\text{Al}(\text{OH})_3$. The control rats were injected with an emulsion containing equal volumes of PBS and $\text{Al}(\text{OH})_3$.

Rats were then divided into four groups, one served as positive control group and other two received nilotinib at the doses of 25 and 50 mg/kg while the fourth one received DSCG (100 mg/kg, i.p.). Fourteen days after the injection of antigen to sensitization or $\text{Al}(\text{OH})_3$ (sham-sensitization), the animals received an intraplantar injection of 10 μg of ovalbumin in the right hind paw, diluted in 100 μl of PBS. Measurement of paw size was carried out using caliper rule (Digimatic, Mitutoyo 500, Japan) before the injection of the inflammatory challenge (time 0) and 2 h after the challenge then the animals were killed by cervical dislocation. The paws were excised and the footpads fixed in 10% neutral buffered formalin solution. Histological specimens were then stained with hematoxylin–eosin (H&E). The tissues were examined under a microscope in a random order and without knowledge of animal or group.

2.5. Passive cutaneous anaphylactic reaction

The experiment was done according to the method described by Coleman et al. (1983) and Matsuda et al., 1997, and modified by Portela et al. (2002). In brief, rats were immunized with 0.5 ml of suspension containing 1 mg of ovalbumin and 300 mg aluminum hydroxide ($\text{Al}(\text{OH})_3$) in normal saline (aluminum-precipitated ovalbumin). Seven days later, rats were immunized again following the same procedure. The aluminum precipitate acts as an adjuvant to promote IgE production. Fourteen days later, rats were anesthetized with ketamine and blood was withdrawn from the carotid artery and left to coagulate

for 20 min; then rat anti-ovalbumin serum was separated. Sera were diluted to the concentration that produced extravasations from 10 to 20 mm in diameter (Park et al., 2002). Diluted sera (100 μl) were intradermally injected into 2 sites on the shaved dorsal skin of non sensitized rats. After 48 h, the animals were challenged with 0.5 ml of a solution containing 2 mg of ovalbumin plus 5 mg of Evans blue via the tail vein (Dai et al., 2002). Nilotinib was administered orally 1 h before the ovalbumin challenge at the doses of 25, 50, and 75 mg/kg. As a control, an anti-histamine and mast cell stabilizing agent, DSCG (100 mg/kg, i.p.), was given 1 h prior to the challenge with ovalbumin. Thirty minutes after the challenge, rats were sacrificed by cervical dislocation and the skin surrounding the spots was removed. The amount of dye was determined quantitatively based on the method of Katayama et al. (1978) after extraction with 1 ml of 1 N KOH and 9 ml of a mixture of 0.6 N phosphoric acid and acetone (5:13 v/v). Absorbance of supernatant was measured at 620 nm using UV–visible spectrophotometer (Model: 1200, 220 V, 50/60 Hz, 90 VA. Unico Instrument Co. LTD., USA). The dye concentration in shaved dorsal skin was determined from the standard curve of known concentrations of Evans blue dye and the results were expressed as $\mu\text{g}/\text{site}$.

2.6. Assay of histamine release

2.6.1. Preparation of rat peritoneal mast cells

Rat peritoneal mast cells were isolated as previously described (Shin et al., 1999). Briefly, rats were anesthetized by ether, and injected with 20 ml of calcium-free HEPES-Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM NaHCO_3 , 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin), into the peritoneal cavity, and the abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. The peritoneal cells were sedimented at $150 \times g$ for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells (i.e. macrophages and small lymphocytes) according to the method described by Yurt et al., 1977. In brief, peritoneal cells were suspended in 1 ml of Tyrode buffer B, layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged at room temperature for 15 min at $400 \times g$. The cells remaining at the buffer–metrizamide interface were aspirated and discarded. Rat peritoneal mast cells preparations were about 95% pure as assessed by toluidine blue staining and at least 98% of these cells were viable as judged by trypan blue exclusion. Mast cells (2×10^5 cells/ml) were resuspended in 1 ml of HEPES-Tyrode buffer (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.0 mM MgCl_2 , 1.4 mM CaCl_2 , 5.6 mM glucose, 0.1% bovine serum albumin).

2.6.2. Sensitization procedure

In experiments where mast cells were challenged with ovalbumin, rats were sensitized with ovalbumin as described by Brooks et al. (1999). Briefly, rats were injected intraperitoneally with 1 ml ovalbumin (10 $\mu\text{g}/\text{ml}$) and $\text{Al}(\text{OH})_3$ (100 mg/ml) on day 0. This injection was repeated on day 12. Peritoneal mast cells were harvested, as described previously, on day 21.

2.6.3. Measurement of histamine

Rat peritoneal mast cells suspensions (2×10^5 cells/ml) were incubated for 15 min at 37°C before the addition of any treatment for stabilization. The cells were pre-incubated without or with nilotinib (5, 10 and 20 μM) for 30 min at 37°C , which covered the mean plasma and peak concentrations observed in humans after standard doses (Kantarjian et al., 2006). In one incubation DSCG (200 μM) was used as a reference inhibitor (Choi and Yan, 2009). After the drug treatment period, the cells were stimulated with further incubation with either compound 48/80 (10 $\mu\text{g}/\text{ml}$) or ovalbumin (100 $\mu\text{g}/\text{ml}$) for 30 min. The reaction was stopped by cooling the tubes in ice. The cells were

separated from the released histamine by centrifugation at $400\times g$ for 5 min at 4 °C. Residual histamine in the cells was released through disrupting the cells with perchloric acid and then centrifugation at $400\times g$ for 5 min at 4 °C (Moon et al., 2007).

The histamine content in the harvested supernatant was measured using the o-phthalaldehyde spectrofluorometric procedure (Shore et al., 1959). The fluorescent intensity was measured at 440 nm (excitation at 360 nm) in spectrofluorometer. The inhibition percentage of histamine release was calculated using the following equation:

$$\text{Inhibition(\%)} = (A - B) \times 100 / A$$

A is histamine release level in control; while B is histamine release level in nilotinib treated cells.

2.7. Assay of TNF- α

Rat peritoneal mast cells (2×10^5 /ml) were suspended in 100 μ l Tyrode's buffer. The cells were sensitized through incubation with ovalbumin (100 μ g/ml) and in the presence or absence of varying doses of nilotinib (5, 10, and 20 μ M) or DSCG (200 μ M) for 30 min. The negative control group was incubated with PBS. The level of TNF- α in the supernatant was determined by using commercial enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions (DuoSet; R&D Systems Inc., Minneapolis, MN). TNF- α was determined from a standard curve.

2.8. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's Kramer multiple comparison test. The values are means $\pm$ S.E.M for six rats in each group. P value < 0.05 was considered as significant.

3. Results

3.1. Effect of nilotinib on the systemic anaphylactic shock induced by compound 48/80

As shown in Table 1, intraperitoneal injection of compound 48/80 (8 mg/kg) produced a fatal anaphylactic shock in all mice. Nilotinib oral pretreatment at concentrations ranging from 5 to 150 mg/kg resulted in a significant reduction in the mortality rate. DSCG (1000 mg/kg, oral) used as positive control drug produced potent inhibition of the compound 48/80-induced systemic anaphylactic shock. In addition, the mortality of mice treated with nilotinib (150 mg/kg) 5, 10, 20, and 30 min after compound 48/80 injection increased in a time-dependent manner (Table 2).

Table 1
Effect of nilotinib on compound 48/80-induced systemic anaphylaxis.

	Dose (mg/kg, IP)	Compound 48/80 (8 mg/kg)	Mortality (%)
Control	None (saline)	+	100
Nilotinib	5	+	70
	10	+	40
	50	+	20
	100	+	10
	150	+	10
	150	—	0
DSCG	1000	+	10

Groups of mice ($n = 10$ /group) were orally administered nilotinib at various doses 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead mice $\times$ 100/total number of experimental mice.

Table 2

Time-dependent effect of nilotinib on compound 48/80-induced systemic anaphylaxis.

	Time	Compound 48/80 (8 mg/kg)	Mortality (%)
Control	—	+	100
Nilotinib (150 mg/kg)	5	+	20
	10	+	40
	20	+	80
	30	+	100

Mice ($n = 10$ /group) were orally administered nilotinib (150 mg/kg) 5, 10, 20 and 30 min after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead mice $\times$ 100/total number of experimental mice.

3.2. Effect of nilotinib on ovalbumin-induced paw edema

The anaphylactic edema caused by ovalbumin (30 μ g) triggered paw swelling within 2 h in sensitized rats. Oral administration of nilotinib (25 or 50 mg/kg) produced a significant reduction in the paw edema formation as compared to the non-treated ovalbumin-sensitized group. The non-sensitized group (saline) did not present footpad edema after ovalbumin challenge (Fig. 1).

Histological examination of the rat paws after ovalbumin challenge revealed neutrophils infiltration and marked inflammation of the muscle cells in the paws injected with ovalbumin. However, the animals that received nilotinib (25 or 50 mg/kg) as well as DSCG treated animals showed normal paw architecture (Fig. 2).

3.3. Effect of nilotinib on passive cutaneous anaphylactic reaction

Passive cutaneous anaphylaxis, induced by an injection of IgE and antigen, is one of the most important in vivo models of local allergic reaction. Oral administration of nilotinib showed a significant inhibition rate in passive cutaneous anaphylactic reaction at the doses 50 mg/kg (P 0.01) and 75 mg/kg (P 0.001) as compared to the control group (Fig. 3). The reference drug DSCG (100 mg/kg, i.p.), a typical anti-allergic drug used as a positive control, also significantly inhibited passive cutaneous anaphylaxis by 68.1% as compared to the control group (Fig. 3).

3.4. Effect of nilotinib on histamine release from rat peritoneal mast cells

High levels of histamine were released from rat peritoneal mast cells when stimulated with either compound 48/80 (10 μ g/ml) or

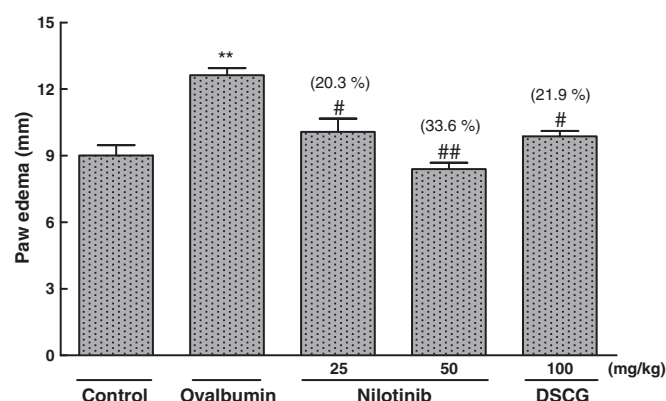


Fig. 1. Effect of nilotinib on ovalbumin-induced paw edema. Nilotinib (25 and 50 mg/kg, oral) and DSCG (100 mg/kg, i.p.) were administered at 1 h prior to the challenge. DSCG was used as a typical anti-allergic control drug. The paw edema was measured in mm 2 h after ovalbumin challenge. The results are expressed as the mean $\pm$ S.E.M. of six independent experiments. ** $P < 0.001$ significantly different compared to the control group (one-way ANOVA). # $P < 0.01$, ## $P < 0.001$, significantly different compared to the ovalbumin group (one-way ANOVA).

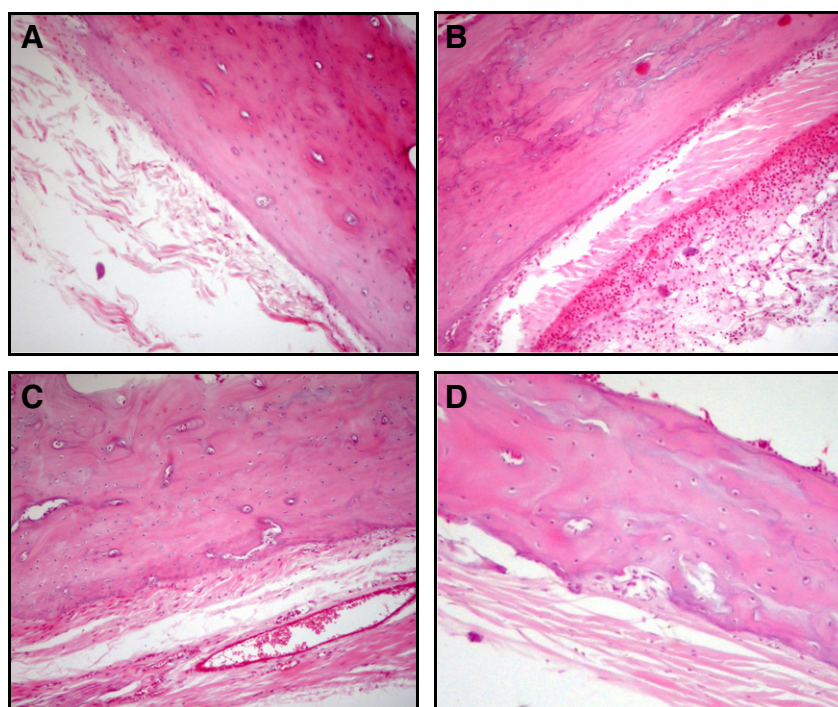


Fig. 2. Effect of nilotinib (25 and 50 mg/kg, oral) on histopathological changes in ovalbumin-induced paw edema experiment, specimen were stained with H&E (100 $\times$). A) Control shows normal architecture of the covering dermal and epidermal layers as well as subcutaneous tissue of the skin; B) ovalbumin shows paw tissue exhibiting large amount of inflammatory leukocyte mainly neutrophils infiltration with edema, C) nilotinib; and D) DSCG show normal architecture of paw and no signs of edema.

ovalbumin (100 μ g/ml). Nilotinib inhibited compound 48/80-induced histamine release from rat peritoneal mast cells in a concentration-dependent manner (5, 10, 20 μ M) as shown in Fig. 4. In particular, nilotinib significantly decreased histamine release at the doses 10 μ M ($P < 0.05$) and 20 μ M ($P < 0.001$) as compared to the control group. Also, DSCG showed a significant inhibition rate ($P < 0.001$) as compared to the control group. In addition, the inhibitory effect of nilotinib and DSCG on ovalbumin-mediated histamine release from rat peritoneal mast cells was shown in Fig. 4. Nilotinib inhibited ovalbumin-mediated histamine release from rat peritoneal mast cells in a concentration-dependent manner.

3.5. Effect of nilotinib on secretion of TNF- α from rat peritoneal mast cells

TNF- α is one of the most important pro-inflammatory cytokines that is involved in allergic diseases. Therefore, the effect of nilotinib on the secretion of TNF- α was tested. As shown in Fig. 5, stimulated rat peritoneal mast cells released significantly more TNF- α ($P < 0.001$) as compared to control group (rat peritoneal mast cells treated with equal amount of PBS only). Treatment with nilotinib resulted in significant suppression of TNF- α secretion from rat peritoneal mast cells in a dose-dependent manner as compared to ovalbumin group ($P < 0.001$).

4. Discussion

Anaphylactic allergic reaction is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators and various cytokines from mast cells which are located throughout the human body. Upon allergen exposure, mast cells are stimulated and degranulated with release of histamine and a vast array of cytokines which diffuses rapidly into the surrounding tissues and exert their inflammatory effects (Jeong et al., 2002; Kemp and Lockey, 2002). Mast cells express in their membrane the tyrosine kinase receptor (KIT) which has an extracellular domain that binds to stem cell factor (SCF). The SCF/KIT pathway has been involved in mast cell development, survival, and function. SCF acts by itself to induce mast cell survival, or in synergism with other growth factors such as IL-3 (Reber et al., 2006). In addition, SCF stimulates mast cell degranulation with release of mediators, such as histamine, and also expression of inflammatory cytokines and chemokines (Ray et al., 2010). It also dramatically enhances antigen-dependent mast cell degranulation, as indicated by studies conducted in both rodent and human mast cells. In the absence of SCF, antigen only minimally increases cytokine message and protein levels in human mast cells, whereas in the presence of SCF, the levels are remarkably elevated (Hundley et al., 2004; Jensen et al., 2008). Also, the IgE-dependent TNF- α and IL-5 release by mast cells is potentiated by SCF (Okayama et al., 1998). Therefore,

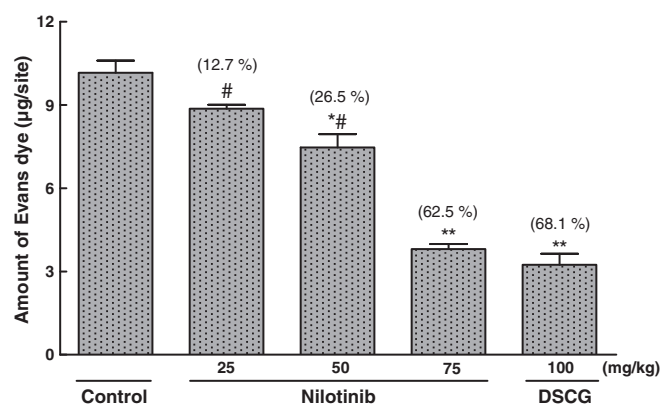


Fig. 3. Effect of nilotinib on passive cutaneous anaphylactic reaction. Nilotinib (25, 50 and 75 mg/kg, oral) and DSCG (100 mg/kg, IP) were administered 1 h prior to the challenge. Diluted rat anti-ovalbumin serum (100 μ l) was intradermally injected into spots on the dorsal skin of rats. After 48 h, rats were challenged with 2 mg of ovalbumin and 5 mg of Evans blue in 0.5 ml normal saline via tail vein. After 30 min, animals were sacrificed, and the amount of dye that leaked into the spots was determined. Inhibition (%) = [(amount of Evans blue without nilotinib - amount of Evans blue with nilotinib) / amount of Evans blue without nilotinib] $\times$ 100. Each amount of Evans blue represents the mean $\pm$ S.E.M. of six independent experiments. ** $P < 0.001$, * $P < 0.01$, significantly different compared to the control group (one way ANOVA). # $P < 0.001$, significantly different compared to the DSCG group (one way ANOVA).

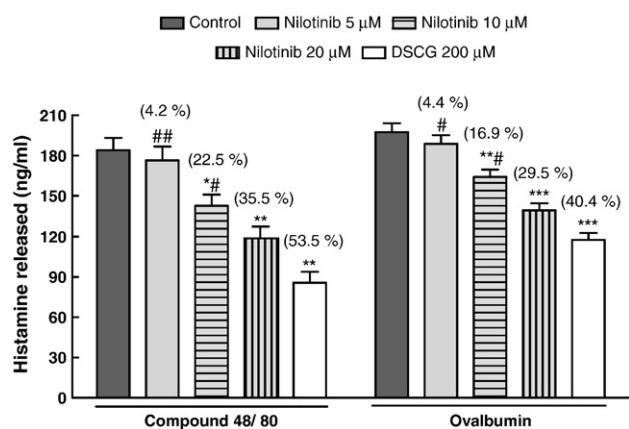


Fig. 4. Effect of nilotinib (5, 10 and 20 μM) on compound 48/80- and ovalbumin-mediated histamine release from rat peritoneal mast cells (RPMCs). RPMCs were pre-incubated with nilotinib at 37 °C for 30 min prior to the incubation with compound 48/80 or ovalbumin. Inhibition (%) = $(A - B) \times 100 / A$ where A is histamine release level in control, while B is histamine release level in nilotinib treated cells. Each data value represents the mean ± S.E.M. of six independent experiments. ***P < 0.001, **P < 0.01, *P < 0.05, significantly different compared to the control group (one way ANOVA). ##P < 0.001, #P < 0.01, significantly different compared to the DSCG group (one way ANOVA).

inhibition of KIT signaling could have beneficial action on the acute effects of mast cell degranulation and production of inflammatory mediators (Ray et al., 2010).

In experimental allergic models, DSCG (a mast cell stabilizer) has shown a potent inhibitory effect on the immediate type allergic reactions in rats. This effect of DSCG is thought to be based on the inhibition of the mediator release from mast cells (Shin et al., 2004). The present study evaluated the inhibitory effects of nilotinib in comparison with those of DSCG. The results have shown a prominent inhibitory effect of nilotinib against immunologic and non-immunologic anaphylactic reactions. Particularly, nilotinib protected against compound 48/80-induced systemic allergic reaction and antigen-mediated local allergic reaction. The ability of nilotinib in neutralizing the action of the inflammatory mediators was clearly evident by its anti-edematous effect against ovalbumin-induced paw edema in rats. Furthermore, nilotinib attenuated compound 48/80-induced or ovalbumin mediated histamine release from rat peritoneal mast cells. Additionally; nilotinib inhibited the production of TNF-α.

It is well known that compound 48/80, a classic secretagogue for mast cells, can induce a mast cell-dependent, non-specific

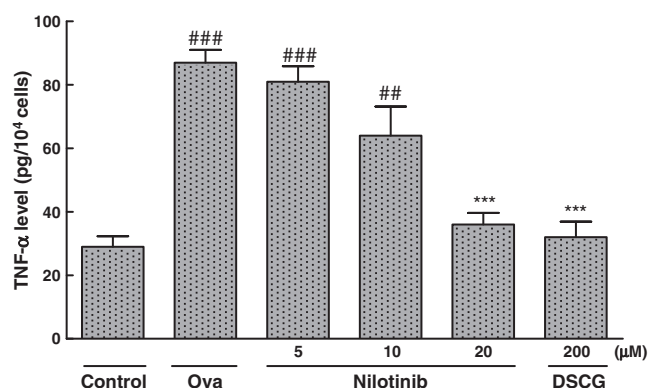


Fig. 5. Inhibitory effect of nilotinib (5, 10 and 20 μM) on the IgE-Mediated production of TNF-α in rat peritoneal mast cells. Each data value represents the mean ± S.E.M. of six independent experiments. ###P < 0.001, ##P < 0.01, significantly different compared to the control group (one way ANOVA). ***P < 0.001, significantly different compared to the Ova group (one way ANOVA).

anaphylactic reaction. Compound 48/80 activates mast cells via phospholipase D and heterotrimeric GTP-binding proteins (Chahdi et al., 2000). It can directly activate trimeric G proteins, primarily those of the Gi and Go categories leading to stimulation of mast cells (Mousli et al., 1990; Shefler and Sagi-Eisenberg, 2001). Activated mast cells either by allergen or compound 48/80 produce pro-inflammatory mediators, including certain cytokines which results in allergic diseases and other physiological effects. Recent reports have demonstrated the role of tyrosine kinases in compound 48/80 mediated degranulation process. The tyrosine kinase inhibitor, genistein, has been reported to inhibit compound 48/80 induced histamine release (Štampelj and Ferjan, 2005). In vitro, compound 48/80 stimulates only certain subtypes of mast cells, such as rat peritoneal mast cells, to induce the rapid release of inflammatory mediators while rat mucosal mast cells and RBL-2H3 cells are not activated by the compound. The results of the present study showed that oral pretreatment with nilotinib produced a significant protection against compound 48/80-induced systemic anaphylaxis in a dose-dependent manner. The inhibitory effect of nilotinib was comparable to that of the clinically available anti-allergic drug, DSCG, which is known as a mast cell stabilizer (Shin et al., 2004). These data suggest that nilotinib can protect from immediate immune response by suppressing the rate of mast cell degranulation and the direct binding to G protein evoked by compound 48/80.

One of the most important approaches used in the examination of the immunopathological mechanisms of anaphylactic and inflammatory disorders is to elicit the formation of paw edema, injecting various substances into the subplantar tissue of rats or mice (Guo et al., 1997). Nilotinib (25, 50 mg/kg) showed a significant inhibition on the hind paw edema in rats caused by ovalbumin. Several studies have showed that 5 min after antigen challenge the mast cells release histamine, proteoglycans (heparin, chondroitin sulfate E) and proteases. Lipid-derived mediators are produced in about 5–30 min, including the arachidonic acid metabolites: prostaglandins, thromboxanes and leucotrienes. All of these mediators contribute to the edema formation in an established acute inflammatory process. Recent studies have provided evidence for the critical involvement of TNF-α in the formation of paw edema in different experimental models (Fernandes et al., 2003; Kalokasidis et al., 2009; Rocha et al., 2006). This has been attributed to the effect of TNF-α on the mechanisms underlying edema formation, namely increased vascular permeability through tyrosine kinase signaling (Page et al., 2009). In this way, nilotinib treatment could be inhibiting the production of the inflammatory mediators responsible for the edema formation or interfering with the mast cell degranulation.

In addition, nilotinib-administered rats are protected from ovalbumin-mediated passive cutaneous anaphylaxis, which is one of the most important in vivo models of anaphylaxis in a local allergic reaction. Furthermore, results of the present study have demonstrated the preventive action of nilotinib against compound 48/80-induced and ovalbumin mediated histamine release from rat peritoneal mast cells. In phase I clinical studies, at the steady-state, mean serum levels of nilotinib were: 1.0 μM at 400 mg/day, 1.7 μM at 800 mg/day, and 2.3 μM at 1200 mg/day; at 800 mg/day, the dose selected for phase II studies, steady state mean serum peak levels of drug were 3.6 μM (Kantarjian et al., 2006). Based upon this, to include the clinically relevant dose range of nilotinib, the in vitro assays described in this study were performed at concentrations between 5 and 20 μM.

Previous studies have shown that stimulation of rat peritoneal mast cells with compound 48/80 or IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Compound 48/80 has been reported to increase the permeability of the lipid bilayer membrane by causing a perturbation of the membrane leading to increase in the membrane permeability which may be an essential trigger for the release of mediators from mast cells (Moon et al., 2007). Therefore, it is reasonable to presume that the anti-anaphylactic action of nilotinib

would be due to its membrane-stabilizing action on mast cells. Also, these results suggest that the protective effect of nilotinib against ovalbumin mediated allergic reaction might be in part due to the activity of nilotinib to inhibit several protein tyrosine kinases in rat peritoneal mast cells. This is consistent with previous investigations which have clearly demonstrated that the tyrosine kinase inhibitor, imatinib, has effectively blocked KIT-mediated mast cells degranulation response as well as KIT-induced cytokine release in mast cells (Jensen et al., 2008).

Although mast cells store small amounts of cytokines in their granules, these cells dramatically increase the production of TNF- α and other cytokines after their surface Fc ϵ RI are cross-linked with specific antigen (Bradding et al., 1993; Galli et al., 1991). To gain further insights into the mechanisms that might be involved in nilotinib mediated protection against experimental allergic models, the level of TNF- α was measured. The results showed marked inhibition of TNF- α secretion in a case of nilotinib treatment. This is in agreement with previous reports that have shown the ability of nilotinib to inhibit TNF- α production both in vivo or in vitro (Akashi et al., 2011). So in the present study, the beneficial effects of nilotinib are likely mediated by inhibition of inflammatory mediators and cytokines produced by mast cells which may result from inhibition of KIT signaling.

5. Conclusions

In summary, the results from this study demonstrate the evidence that nilotinib inhibits the immunologic and nonimmunologic immediate-type allergic reactions in experimental murine models.

Conflict of interest

The author declares no conflict of interest.

Acknowledgments

The author acknowledges Dr. Abdelhadi M. Shebl, (Department of Pathology, Faculty of Medicine, University of Mansoura, Mansoura, Egypt) for histopathological evaluation and Dr. Paul Manley (Novartis Pharma, Basel, Switzerland) for providing nilotinib.

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