ORIGINAL CONTRIBUTION

Rhinology, 49, 100-106, 2011

Syk-kinase inhibition prevents mast cell activation in nasal polyps*

Joke Patou[1], Gabriele Holtappels[1], Karen Affleck[2], Paul Van Cauwenberge[1], Claus Bachert[1]

[1] Upper Airways Research Laboratory, Department of Otorhinolaryngology, Ghent University, Ghent, Belgium
[2] GSK, Stevenage, United Kingdom

SUMMARY

Background: *Mast cells are crucial effector cells in the allergic cascade. The cross-linking of the high affinity IgE receptor (FcεRI) activates mast cells and basophils. Spleen tyrosine kinase (Syk) is positioned upstream of the IgE receptor signal transducing pathway and may represent an important target for the treatment of nasal inflammatory diseases.*

Objective: *We measured effects of a specific Syk inhibitor in the release of mast cell mediators in human cord blood-derived mast cells (CBDMCs) (in-vitro) and in human nasal tissue (ex-vivo).*

Methods: *Surgical samples were collected from patients with nasal polyposis who underwent sinus surgery. Tissue cubes of ± 0.9 mm³ were primed with myeloma IgE (1 µg/ml), preincubated with Syk inhibitor NVP-QAB205 in different concentrations and then stimulated with tissue culture medium, anti-IgE 10 µg/ml and anti-IgE 30 µg/ml. Supernatants were analysed for concentrations of histamine, LTC4/LTD4/LTE4 and PGD_2. CBDMCs were likewise pre-incubated with compound, prior to stimulation with anti-IgE at 10 µg/ml.*

Results: *In CBDMCs, the Syk inhibitor prevented degranulation assessed by measurement of histamine release and the production of $LTC_4/LTD_4/LTE_4$ and PGD_2. Furthermore, the Syk inhibitor was similarly able to significantly inhibit the release of these granules and newly synthesized mediators by nasal polyp mast cells in a dose dependent manner.*

Conclusion: *Although the critical role of Syk in the IgE receptor signal transduction pathway has been well documented in vitro, this study supports the importance of Syk in IgE receptor-mediated degranulation of mast cells ex-vivo within nasal tissue. Thus, inhibition of Syk may represent an important therapeutic strategy for the treatment of upper airway disease with mast cell involvement, such as allergic rhinitis.*

Key words: Syk inhibition, nasal polyps, mast cell, IgE receptor

INTRODUCTION

Mast cells are multifunctional effector cells of the immune system and play a crucial role in the allergic response. Activation of these cells induces the release of a wide collection of mediators that initiate the cascade of clinical symptoms associated with allergic rhinitis, such as itching, sneezing, rhinorrhea and nasal obstruction [(1)]. First of all, there is the release of preformed inflammatory mediators, such as histamine and tryptase localized in specialized granules, and secondly there is the *de novo* synthesis and secretion of cytokines, chemokines, and eicosanoids [(1,2)]. During the acute allergic reaction, there is mainly the release of preformed mediators such as histamine but also the release of newly formed leukotrienes ($LTC_4/D_4/E_4$) and prostaglandin D2 (PGD_2) [(3)].

The IgE/allergen – mediated stimulation through the cross-linking of the high-affinity receptor for IgE (FcεRI) remains a very important step in the activation of the mast cell. The FcεRI expressed on mast cells and basophils is a tetrameric receptor comprised of the IgE-binding α chain, the signal-amplifying β chain [(4)] and the two signal transducing γ chains [(5)]. The transmission of the signals is dependent on the specific sequence of the immunoreceptor tyrosine-based activation motif (ITAM) present on the β and γ chains. After the phosphorylation of the ITAMs by the protein tyrosine kinase Lyn, there is the activation of spleen tyrosine kinase Syk through ITAM binding [(6,7)]. Furthermore there is the activation of several adaptor proteins and transcriptional factors. The activation of FcεRI finally leads to degranulation, cytokine and eicosanoid production of mast cells.

*Received for publication: September 5, 2009; accepted: May 14, 2010

DOI:10.4193/Rhino09.147

Consequently, as Syk is positioned upstream in the cell signalling pathway, therapies targeting Syk should block three mast cell functions: the release of preformed mediators such as histamine, the production of lipid mediators such as leukotrienes and prostaglandins and the secretion of cytokines [8].

We have demonstrated before [9] that nasal polyp tissue can be used for testing the early phase of an allergic reaction. As large amounts of nasal polyp tissue are easier to access than inferior turbinate tissue, and as nasal polyps and inferior turbinate tissue react in the same concentration-dependent manner with the release of histamine, leukotrienes and PGD_2 to IgE- dependent triggers, we here use mast cell stimulation of nasal polyp tissue to study the effect of Syk inhibition of the allergic early phase reaction.

The essential role of Syk has been proven in Syk-deficient murine mast cells, which are defective in receptor-induced degranulation, cytokine synthesis and intracellular signalling-pathway activation [10]. Piceatannol, originally described as a Syk-selective inhibitor has been found to prevent mast cell degranulation in chopped lung fragments in guinea pigs [11] and in human lung mast cells [12]. Nowadays, the more selective third-generation Syk inhibitors are used, resulting in more confident interpretation of results. One study demonstrated inhibition of histamine release in human basophils and cultured CD-34+ mast cells, and showed inhibition of activation of human bronchial mast cells, assessed as less bronchial smooth muscle contraction in human isolated bronchial preparations. In this study a third-generation specific Syk inhibitor (NVP-QAB205) was used [13].

Syk may represent an important target for the treatment of allergic rhinitis [14]. Since there is currently no data supporting this hypothesis, the aim of this study was to investigate the ability of NVP-QAB205 to inhibit the release of acute phase mediators in nasal polyp tissue after the stimulation of the high affinity IgE receptor.

MATERIALS AND METHODS

Patients

Nasal polyp samples of 8 patients were collected at the Department of Otorhinolaryngology, Ghent University Hospital. The ethical committee of the Ghent University Hospital approved the study and informed consent was obtained from all subjects prior to inclusion in the study. None of the subjects used any intranasal corticosteroids within 1 week prior to surgery, nor took any oral and/or intramuscular corticosteroids within 4 weeks prior to surgery, nor antihistamines or anti-leukotrienes within 4 days prior to surgery, nor oral and intranasal decongestants or intranasal anticholinergics within 2 days prior to surgery. For the female subjects there was no current pregnancy or lactation.

Samples were collected during functional endoscopic sinus surgery from 8 patients (median age 38.5 years, ranging from 20 to 54 years old, 4 female and 4 male patients). Nasal polyposis was diagnosed based on symptoms, clinical examination, nasal endoscopy, and sinus computed tomography (CT) scan according to the EP^{3}OS guidelines [15].

The atopic status of all patients was evaluated by using skin prick tests with a standard panel of inhalant allergens. The reaction to a skin prick test was considered positive if the wheal area caused by the allergen was greater than 7 mm^2 (diameter > 3 mm). Negative and positive controls (10 mg/ml histamine solution) were also included with each skin prick test. In the nasal polyp group, there were 2 patients with an atopic status. None of the patients had asthma or aspirin intolerance in history.

The nasal tissue collected during surgery was immediately transported to the laboratory for the *ex-vivo* stimulations.

Syk inhibitor NVP-QAB205

The Syk inhibitor used in these studies (NVP-QAB205) was a kind gift from GlaxoSmithKline (Stevenage, United Kingdom) and its characteristics have been published [16]. In isolated enzyme assays, this compound has an IC50 of 0.01 µM; hence at the concentrations tested here, it is a selective inhibitory tool.

Mechanical disruption and stimulations of human nasal tissue

Human nasal mucosa and submucosa was cut thoroughly in tissue culture medium consisting of RPMI 1640 (Sigma-Aldrich, Bornem, Belgium), containing 2 mM L-Glutamine (Invitrogen, Merelbeke, Belgium), antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin) (Invitrogen) and 0.1% Bovine Serum Albumin (BSA), (Sigma). The tissue was passed through a mesh to achieve comparable fragments. The tissue fragments (± 0.9 mm^3) were weighed and resuspended as 0.04 g tissue/ 1 ml tissue culture medium.

Tissue was preincubated for 1 hour at 37°C, 5% CO_2 with 1 µg/ml human myeloma IgE (Calbiochem, VWR International, Leuven, Belgium) and washed, tissue fragments were resuspended in the appropriate amount of culture medium, and 0.5 ml of the fragment suspension was dispensed per well in a 48-well plate (BD Falcon). The fragment suspensions were stimulated with either culture medium, or the Syk inhibitor NVP-QAB205 at concentrations of 10^{-8} M, 10^{-7} M, 10^{-6} M and 10^{-5} M for 1 hour. The DMSO concentration was kept constant at 0.1%. Following incubation with the inhibitor, the fragments were stimulated with either tissue culture medium (negative control), or ε-chain specific anti-human IgE antibody (Dako Belgium NV, Heverlee, Belgium) at 10 or 30 µg/ml for 30 minutes. All stimulations were done in duplicate.

Supernatants were separated by centrifugation and stored immediately at -20°C until analysis of histamine, $LTC_4/D_4/E_4$ and PGD_2.

*Cord blood-derived mast cell culture and activation as*say

Cord blood-derived mast cells were differentiated from CD34+ progenitor cells (AllCells, Berkeley, CA, USA), by culturing with stem cell factor (SCF), IL-6 and IL-10 essentially as described by Ochi et al. [17].

Table 1. Overview of the effect of increasing concentrations of the Syk inhibitor on anti-IgE-induced release of histamine (ng/ml), $LTC_4/LTD_4/LTE_4$ (ng/ml) and PGD_2 (pg/ml) in the nasal polyp group (n = 8). Data are expressed as mean ± SD.

Stimulus	release	$+10^{-8}$ M syk inh	$+10^{-7}$ M syk inh	$+10^{-6}$ M syk inh	$+10^{-5}$ M syk inh
Histamine (ng/ml)					
Baseline	24.4 +/– 11.9	23.8 +/– 11.9	22.2 +/– 12.3	21.1 +/– 11.6	22.8 +/– 11.6
Anti–IgE 10µg/ml	42.3 +/– 16.8	37.0 +/– 16.7	32.2 +/– 14	28.1 +/– 14.3	29.7 +/– 13.4
*p-value**		*0.0156*	*0.0078*	*0.0156*	*0.0156*
Anti-IgE 30µg/ml	63.0 +/– 20.6	51.6 +/– 24	39.2 +/– 15.3	36.4 +/–12.0	39.5 +/– 16.4
*p-value**		*0.1484*	*0.0078*	*0.0078*	*0.0078*
$LTC_4/LTD_4/LTE_4$ (ng/ml)					
Baseline	0.08 +/– 0.04	0.07 +/– 0.03	0.07 +/– 0.04	0.06 +/– 0.03	0.07 +/– 0.04
Anti-IgE 10 µg/ml	0.69 +/– 0.59	0.53 +/– 0.46	0.16 +/– 0.14	0.08 +/– 0.02	0.08 +/– 0.06
*p-value**		*0.0391*	*0.0078*	*0.0078*	*0.0078*
Anti-IgE 30 µg/ml	0.92 +/– 0.55	0.66 +/– 0.63	0.17 +/– 0.16	0.07 +/– 0.04	0.08 +/– 0.05
*p-value**		*0.0234*	*0.0078*	*0.0078*	*0.0078*
PGD_2 (pg/ml)					
Baseline	168.0 +/– 163.6	169.3 +/– 105.6	150.4 +/– 96.1	91.1 +/– 70.9	113.3 +/– 52.2
Anti-IgE 10 µg/ml	2662.3 +/– 1793.3	1805.9 +/– 1170.7	1200.5 +/– 918.7	454.2 +/– 284.2	422.4 +/– 291.6
*P-value**		*0.0078*	*0.0078*	*0.0078*	*0.0078*
Anti-IgE 30 µg/ml	5205.5 +/– 3575.5	3631.4 +/– 2543.6	2118.5 +/– 1566.9	1022.4 +/– 192.3	775.6 +/– 306.7
*p-value**		*0.0078*	*0.0078*	*0.0078*	*0.0078*

* versus Anti-IgE stimulation (Statistical analysis; Wilcoxon-test)

The medium used was RPMI 1640 (Sigma) containing 10% heat inactivated CELLect Fetal Bovine Serum (MP Biomedicals), 1% (0.1 mM) non-essential amino acids, 2 mM L-Glutamine, 100 U/ml Penicillin, 100 µg/ml Streptamycin, 10 µg/ml Gentamicin, 200 nM 2-mercaptoethanol (all from InVitrogen), and 100 ng/ml SCF, 50 ng/ml IL-6, and 10 ng/ml IL-10 (all from Peprotech) for 12 weeks.

For inhibitor assays (n = 4), cells were plated at 5 x 104 cells/well in V-bottom 96-well tissue culture plates (Corning), and primed for 6 days 37°C, 5% CO_2 in complete medium containing 10 ng/ml IL-4 (Peprotech) and 2 µg/ml IgE (1 µg/ml kappa, 1 µg/ml lambda; Biodesign, AMS Biotechnology). Cells were then washed in assay buffer (RPMI containing additives as above but with only 4% FCS) and resuspended in 90 µl of assay buffer per well. The Syk inhibitor was added in 10 µl of medium to give final concentrations ranging between 10^{-9} M and 3 x 10^{-7} M, and incubated for 30 minutes at 37°C, 5% CO_2. Anti-IgE antibody (Sigma) was then added in 10 µl to give a final concentration of 10 µg/ml and the plate was incubated for a further 30 minutes. The plate was then centrifuged at 1500 rpm in a bench centrifuge for 5 minutes to pellet cells, and supernatants were transferred to a new plate, and centrifuged again that the supernatants were cell-free.

Determination of mediator concentrations in supernatants

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to measure concentrations of histamine (IBL, Germany), $LTC_4/D_4/E_4$ (Oxford Biomedical Research, Nuclilab BV, Ede, The Netherlands) and PGD_2 (Cayman Chemicals, Ann Arbor, MI, USA).

Statistical analysis

Statistical analysis was performed using the Wilcoxon test (for paired comparisons). P values of less than 0.05 were considered as statistically significant.

RESULTS

Effect of Syk inhibitor on mediator release from CBDMCs

Pre-incubation for 30 minutes with inhibitor resulted in significant inhibition of the release of histamine (Figure 1A, IC50 27.5 nM), leukotrienes (Figure 1B, IC50 22.5 nM) and PGD_2 (Figure 1C, IC50 26.6 nM) in response to IgE receptor cross-linking induced by anti-IgE antibody. Here, the inhibitory potencies of the Syk inhibitor on the different mediators were similar. These efficacies correlated well with the activity of the inhibitor in *in vitro* kinase assays.

Effect of Syk inhibitor on mediator release from human nasal tissue

Stimulation of nasal polyp tissue (n = 8) with anti-IgE (10 µg/ml and 30 µg/ml) resulted in a dose-dependent release of histamine, cysteïnyl leukotrienes and PGD_2 (Table 1).

Effect of Syk inhibitor on histamine release

Increasing concentrations of Syk inhibitor (10^{-8} M, 10^{-7} M, 10^{-6} M and 10^{-5} M) significantly inhibited the release of histamine induced by 10 µg/ml anti-IgE ($p < 0.02$, $p < 0.01$, $p < 0.02$ and $p < 0.02$, respectively) (IC50 13.6 nM). Also the nasal polyp tissue stimulated with 30 µg/ml anti-IgE and preincubated with the Syk inhibitor, showed a significant decrease of histamine release, except for 10^{-8} M Syk inhibitor ($p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) (IC50 13.6 nM) (Table 1 and Figure 2 A).

Effect of Syk inhibitor on $LTC_4/D_4/E_4$ release

In nasal polyps increasing concentrations of Syk inhibitor

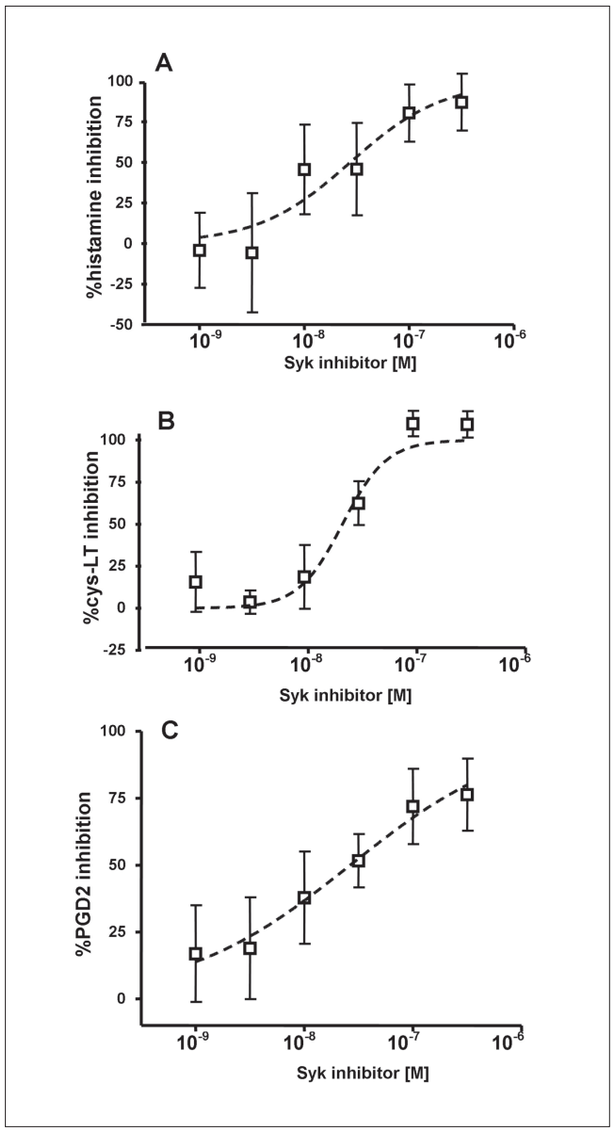

Figure 1. Percent inhibition of mediator release after anti-IgE (10 µg/ml) stimulation and increasing NVP-QAB205 concentrations in cord blood derived mast cells. A) Inhibition of histamine release. B) Inhibition of $LTC_4/D_4/E_4$ release. C) Inhibition of PGD2 release. Results are expressed as mean ± SEM.

gave significant increasing inhibition of $LTC_4/D_4/E_4$ release compared to anti-IgE 10µg/ml stimulation alone ($p < 0.05$, $p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) (IC50 5,7 nM) and compared to anti-IgE 30 µg/ml stimulation alone ($p < 0.05$, $p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) (IC50 3,9 nM) (Table 1 and Figure 2 B).

Effect of Syk inhibitor on PGD_2 release

Furthermore, in nasal polyps the release of PGD_2 was almost completely blocked after preincubation with increasing concentrations of Syk inhibitor ($p < 0.01$, $p < 0.01$, $p < 0.01$ and $p < 0.01$) in comparison with the release after stimulation with anti-IgE 10 µg/ml alone (IC50 80 nM). Also the nasal polyp tissue stimulated with 30 µg/ml anti-IgE showed a significant dose-dependent decrease of PGD_2 release ($p < 0.01$, $p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) (IC50 88 nM) (Table 1 and Figure 2 C).

DISCUSSION

The involvement of mast cells in upper airway disease such as allergic rhinitis is well established, but the current treatment modalities, such as anti-histamines, anti-leukotrienes, and intranasal corticosteroids, are aimed at preventing the activity of single mediators, whereas the mast cell, upon activation by allergen, is able to produce a large number of inflammatory mediators whose activities will be unaffected by such drugs. There is therefore scope to improve on current treatments. One such approach to improve upper airway disease therapies could be the discovery of potent and efficacious mast cell stabilisers, which would effectively block the production of both early mediators (e.g. histamine, cysteinyl-leukotrienes, PGD_2) and cytokines, which should relieve both immediate symptoms experienced on exposure to IgE-dependent triggers, but also halt the subsequent recruitment of inflammatory cells into the nasal mucosa. An understanding of the signalling pathways initiated upon cross-linking of the high affinity IgE receptor has helped identify potential future drug targets, and an example of one such protein is the tyrosine kinase Syk which appears to be critical for mast cell function.

To evaluate the mast cell stabilisation potential of novel molecules such as Syk inhibitor NVP-QAB205, inhibition of mediator release of mast cells was here studied in cord blood derived mast cells and in nasal polyps. Our group recently developed a human nasal mucosal stimulation model with anti-IgE stimulations that mimics the allergic acute phase reaction [9]. Stimulations with anti-IgE on IgE-primed nasal tissue fragments lead to a dose-dependent release of histamine, leukotrienes and PGD_2 both in inferior turbinates and nasal polyps. In view of the mediators and the time course after stimulation, those outcome measures are specific for a mast cell response. As inferior nasal turbinate tissue in allergic rhinitis, nasal polyp tissue is characterized by the presence of effector cells such as mast cells and eosinophils, both typical for allergic inflammation [18]. As nasal polyps and inferior turbinate tissue react in the same concentration-dependent manner to IgE-dependent triggers, and nasal polyp tissue is easily provided by surgical procedures performed to remove disease, nasal polyp tissue was used in this pharmacological profiling setting [9].

Syk is not only expressed in mast cells [19,20] and basophils [21], but also in eosinophils [22], neutrophils [23], macrophages [24], dendritic cells [25] and B-cells [26]. Syk has an essential role in immunoreceptor signalling. Allergen-specific IgE binds to the surface of mast cells and basophils through high affinity Fc receptors for IgE. Following subsequent allergen exposure, activation of mast cells and basophils through crosslinking of the tetrameric FcεRI occurs and causes degranulation and *de*

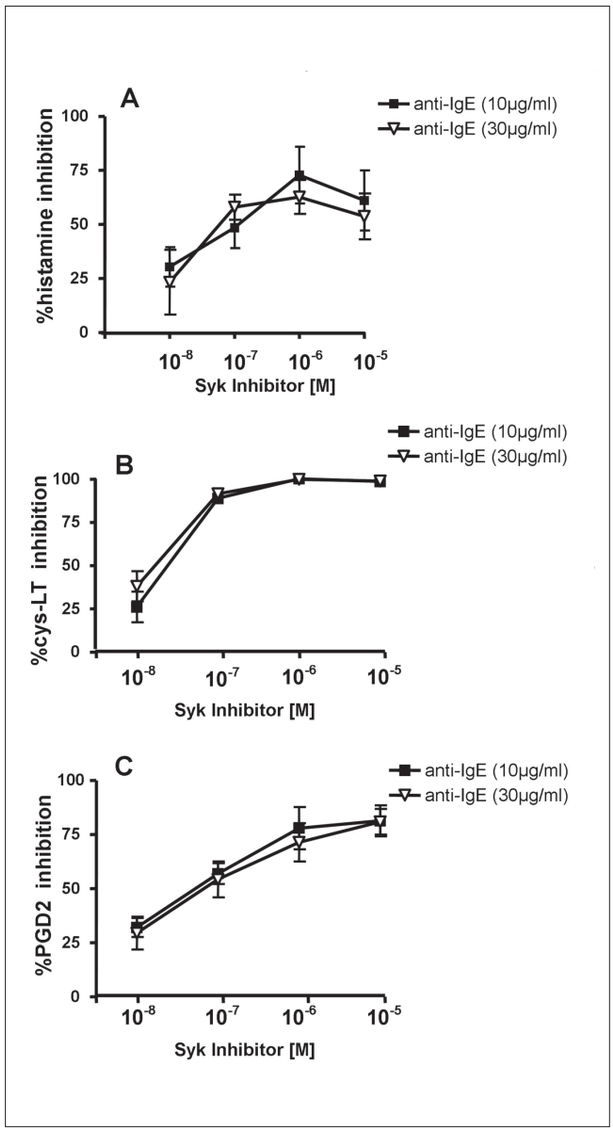

Figure 2. Percent inhibition of mediator release after anti-IgE 10µg/ml(■) and anti-IgE 30 µg/ml (▽) stimulation and increasing NVP-QAB205 concentrations in nasal polyp tissue. A) Inhibition of histamine release. B) Inhibition of LTC4/D4/E4 release. C) Inhibition of PGD2 release. Results are expressed as mean ± SEM.

novo synthesis and release of mediators, all important in the allergic inflammation cascade. Receptor crosslinking results in the recruitment and the activation of cytoplasmatic kinase Lyn. Lyn causes tyrosine phosphorylation of ITAM in the cytoplasmatic domains of β- and γ-chains. Phosphorylated ITAM in the γ-chains serve as binding sites for Syk SH2 domains. As a result of its binding to ITAM, Syk becomes activated [7,10,27]. Activated Syk regulates multiple intracellular signalling pathways. Some important downstream targets of Syk in mast cells include phospholipase Cγ (PLCγ), which may lead to mast cell degranulation, and the mitogen-activated protein kinase (MAPK) pathway which in turn may lead to leukotrienes and prostaglandine production [8].

There is a growing body of literature of the importance of Syk in the development of allergic inflammation in the lower airways. In animal asthma models, aerosolized Syk antisense oligonucleotides (ASO) inhibited many of the central components (f.e. the level of eosinophils, tumour necrosis factor) of allergic asthma [28], and a Syk-selective tyrosine kinase inhibitor prevented mast cell degranulation and airway hyperresponsiveness [11,29]. In human lung mast cells piceatannol prevented histamine release [12]. Piceatannol was originally described as a Syk-specific inhibitor but it inhibits other kinases as well [30] and applied at concentrations inhibiting IgE-induced mediator release from basophils, it doesn't act on Syk [31].

Syk inhibitor NVP-QAB205 is considered selective for its respective kinase as it doesn't inhibit other signaling steps thought to be downstream of Syk kinase such as Ras-Erk pathway elements. It is known to have an IC50 of 40 nM in human basophils and inhibition of Syk activity has no influence on either loss from the cell surface or degradation of the IgE receptor. Furthermore, it inhibits histamine release in cultured mast cells and it inhibits bronchial smooth muscle contraction [13,32].
Supplementary with the limited findings of Syk inhibition in the human bronchial biopsies, this study reports the potency of NVP-QAB205 to inhibit the release of acute phase mediators in human nasal tissue. The inhibitor was able to completely and potently inhibit mediator release from cord blood derived mast cells, thus enabling a comparison of its effect in nasal tissue fragments.

In nasal polyps, the Syk inhibitor significantly inhibited the release of histamine, $LTC_4/D_4/E_4$ and PGD_2. The IC50 values (13 nM for histamine, about 4.8 nM for leukotrienes and 80 nM for PGD_2) were comparable to the IC50 value of histamine in human basophils. In CBDMCs, the potencies of the Syk inhibitor on the different mediators are similar (pLTs = histamine = PGD_2) however in nasal polyps they are different (pLTs > histamine > PGD_2); probably due to the more complex physiological and disease-relevant system in human tissue. These are all mast cell products that may be detected immediately after exposure to allergens, causing typical symptoms such as rhinorrhea, sneezing, itching and nasal obstruction [1]. Furthermore, through the release of various inflammatory mediators, mast cells may contribute to the induction of eosinophilic inflammation and to the formation and progression of nasal polyps [33]. In nasal fluids from patients with nasal polyps, histamine levels are significantly higher than those observed in patients without nasal polyps [34]. In nasal polyps, preincubation with the Syk inhibitor gave a 100% inhibition of the cysLTs production after anti-IgE stimulation and there was an almost total (± 80%) inhibition of PGD_2 production. There was also a significant decrease of histamine release; however the highest inhibition here was around 60%.

The inhibition of the release of these mediators seemed to be dose-dependent.

A clinical study, where another specific Syk inhibitor (R112) was applied, demonstrated the reduction of symptoms in seasonal allergic rhinitis patients [35]. Our results suggest that this could be due to the inhibition of histamine, cysLTs and PGD_2.

SUMMARY

Syk activation is an important upstream event in the pathways activated through FcεRI and may therefore control the synthesis and release of a whole range of mediators of both the early and late allergic responses. This study demonstrates that Syk inhibition may prevent the degranulation of and the *de novo* synthesis in mast cells and in human nasal tissue. In future, specific Syk inhibitors might provide a new therapeutic possibility in the treatment of upper airway disease with mast cell involvement such as allergic rhinitis.

REFERENCES

1. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001; 108: S147-334.
2. Durham SR. The inflammatory nature of allergic disease. Clin Exp Allergy 1998; 28 Suppl 6: 20-24.
3. Hansen I, Klimek L, Mosges R, et al. Mediators of inflammation in the early and the late phase of allergic rhinitis. Curr Opin Allergy Clin Immunol 2004; 4: 159-163.
4. Lin S, Cicala C, Scharenberg AM, et al. The Fc(epsilon)RIbeta subunit functions as an amplifier of Fc(epsilon)RIgamma-mediated cell activation signals. Cell 1996; 85: 985-995.
5. Sakurai D, Yamasaki S, Arase K, et al. Fc epsilon RI gamma-ITAM is differentially required for mast cell function in vivo. J Immunol 2004; 172: 2374-2381.
6. Chen T, Repetto B, Chizzonite R, et al. Interaction of phosphorylated FcepsilonRIgamma immunoglobulin receptor tyrosine activation motif-based peptides with dual and single SH2 domains of p72syk. Assessment of binding parameters and real time binding kinetics. J Biol Chem 1996; 271: 25308-25315.
7. Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. J Allergy Clin Immunol 2006; 117: 1214-1225; quiz 26.
8. Wong BR, Grossbard EB, Payan DG, et al. Targeting Syk as a treatment for allergic and autoimmune disorders. Expert Opin Investig Drugs 2004; 13: 743-762.
9. Patou J, Holtappels G, Affleck K, et al. Enhanced release of IgE-dependent early phase mediators from nasal polyp tissue. J Inflamm (Lond) 2009; 6: 11.
10. Costello PS, Turner M, Walters AE, et al. Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells. Oncogene 1996; 13: 2595-2605.
11. Seow CJ, Chue SC, Wong WS. Piceatannol, a Syk-selective tyrosine kinase inhibitor, attenuated antigen challenge of guinea pig airways in vitro. Eur J Pharmacol 2002; 443: 189-196.
12. Lavens-Phillips SE, Mockford EH, Warner JA. The effect of tyrosine kinase inhibitors on IgE-mediated histamine release from human lung mast cells and basophils. Inflamm Res 1998; 47: 137-143.
13. MacGlashan D, Jr., Undem BJ. Inducing an anergic state in mast cells and basophils without secretion. J Allergy Clin Immunol 2008; 121: 1500-1506, 6 e1-4.
14. Ulanova M, Duta F, Puttagunta L, et al. Spleen tyrosine kinase (Syk) as a novel target for allergic asthma and rhinitis. Expert Opin Ther Targets 2005; 9: 901-921.
15. Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2007. Rhinol Suppl 2007: 1-136.
16. Collingwood SP HJ, Le Grand DM, Mattes H, et al. Preparation of anilinopurine derivatives as inhibitors of tyrosine protein kinase syk. PCT, Int. Appl. Canada, Novartis, 2001.
17. Ochi H, Hirani WM, Yuan Q, et al. T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. J Exp Med 1999; 190: 267-280.
18. Stoop AE, van der Heijden HA, Biewenga J, et al. Eosinophils in nasal polyps and nasal mucosa: an immunohistochemical study. J Allergy Clin Immunol 1993; 91: 616-622.
19. Benhamou M, Gutkind JS, Robbins KC, et al. Tyrosine phosphorylation coupled to IgE receptor-mediated signal transduction and histamine release. Proc Natl Acad Sci USA 1990; 87: 5327-5330.
20. Hutchcroft JE, Geahlen RL, Deanin GG, et al. Fc epsilon RI-mediated tyrosine phosphorylation and activation of the 72-kDa protein-tyrosine kinase, PTK72, in RBL-2H3 rat tumor mast cells. Proc Natl Acad Sci USA 1992; 89: 9107-9111.
21. Kepley CL, Wilson BS, Oliver JM. Identification of the Fc epsilonRI-activated tyrosine kinases Lyn, Syk, and Zap-70 in human basophils. J Allergy Clin Immunol 1998; 102: 304-315.
22. Yousefi S, Hoessli DC, Blaser K, et al. Requirement of Lyn and Syk tyrosine kinases for the prevention of apoptosis by cytokines in human eosinophils. J Exp Med 1996; 183: 1407-1414.
23. Yan SR, Huang M, Berton G. Signaling by adhesion in human neutrophils: activation of the p72syk tyrosine kinase and formation of protein complexes containing p72syk and Src family kinases in neutrophils spreading over fibrinogen. J Immunol 1997; 158: 1902-1910.
24. Darby C, Geahlen RL, Schreiber AD. Stimulation of macrophage Fc gamma RIIIA activates the receptor-associated protein tyrosine kinase Syk and induces phosphorylation of multiple proteins including p95Vav and p62/GAP-associated protein. J Immunol 1994; 152: 5429-5437.
25. Sedlik C, Orbach D, Veron P, et al. A critical role for Syk protein tyrosine kinase in Fc receptor-mediated antigen presentation and induction of dendritic cell maturation. J Immunol 2003; 170: 846-852.
26. Hutchcroft JE, Harrison ML, Geahlen RL. Association of the 72-kDa protein-tyrosine kinase PTK72 with the B cell antigen receptor. J Biol Chem 1992; 267: 8613-8619.
27. Kovarova M, Rivera J. A molecular understanding of mast cell activation and the promise of anti-allergic therapeutics. Curr Med Chem 2004; 11: 2083-2091.
28. Stenton GR, Ulanova M, Dery RE, et al. Inhibition of allergic inflammation in the airways using aerosolized antisense to Syk kinase. J Immunol 2002; 169: 1028-1036.
29. Matsubara S, Li G, Takeda K, et al. Inhibition of spleen tyrosine kinase prevents mast cell activation and airway hyperresponsiveness. Am J Respir Crit Care Med 2006; 173: 56-63.
30. Law DA, Nannizzi-Alaimo L, Ministri K, et al. Genetic and pharmacological analyses of Syk function in alphaIIbbeta3 signaling in platelets. Blood 1999; 93: 2645-2652.
31. Miura K, Lavens-Phillips S, MacGlashan DW, Jr. Piceatannol is an effective inhibitor of IgE-mediated secretion from human basophils but is neither selective for this receptor nor acts on syk kinase at concentrations where mediator release inhibition occurs. Clin Exp Allergy 2001; 31: 1732-1739.
32. MacGlashan D, Jr., Vilarino N. Nonspecific desensitization, functional memory, and the characteristics of SHIP phosphorylation following IgE-mediated stimulation of human basophils. J Immunol 2006; 177: 1040-1051.
33. Pawankar R. Nasal polyposis: an update: editorial review. Curr Opin Allergy Clin Immunol 2003; 3: 1-6.
34. Di Lorenzo G, Drago A, Esposito Pellitteri M, et al. Measurement of inflammatory mediators of mast cells and eosinophils in native nasal lavage fluid in nasal polyposis. Int Arch Allergy Immunol 2001; 125: 164-175.
35. Meltzer EO, Berkowitz RB, Grossbard EB. An intranasal Syk-kinase inhibitor (R112) improves the symptoms of seasonal allergic rhinitis in a park environment. J Allergy Clin Immunol 2005; 115: 791-796.

Joke Patou MD
Upper Airways Research Laboratory
Ghent University Hospital
De Pintelaan 185
B-9000 Ghent
Belgium

Tel: +32-(0)9-240 2332
Fax: +32-(0)9-240 4993
E-mail: Joke.Patou@Ugent.be