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Inhibition of Allergen-Induced Pulmonary Responses by the Selective Tryptase Inhibitor 1,5-bis-{4-[(3-Carbamimidoyl-benzenesulfonylamino)-methyl]-phenoxy}-pentane (AMG-126737)

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ABSTRACT. Emerging evidence suggests that mast cell tryptase is a therapeutic target for the treatment of asthma. The effects of this serine protease are associated with both pathophysiologic pulmonary responses and pathologic changes of the asthmatic airway. In this study, the tryptase inhibitor 1,5-bis-{4-[(3-carbamimidoyl-benzenesulfonylamino)-methyl]-phenoxy}-pentane (AMG-126737) was evaluated for its pharmacologic effects against allergen-induced airway responses. AMG-126737 is a potent inhibitor of human lung mast cell tryptase (K_i = 90 nM), with greater than 10- to 200-fold selectivity versus other serine proteases. Intratracheal administration of AMG-126737 inhibited the development of airway hyperresponsiveness in allergen-challenged guinea pigs with an ED_{50} of 0.015 mg/kg. In addition, the compound exhibited oral activity in the guinea pig model. The *in vivo* activity of AMG-126737 was confirmed in a sheep model of allergen-induced airway responses, where the compound inhibited early and late phase bronchoconstriction responses and the development of airway hyperresponsiveness. These results support the proposed role of tryptase in the pathology of asthma and suggest that AMG-126737 has potential therapeutic utility in this pulmonary disorder. BIOCHEM PHARMACOL **58**;12:1989–1996, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. tryptase; asthma; pulmonary responses; inhibition; AMG-126737

β-Tryptase is a homotetrameric serine protease of human lung mast cells [1, 2]. The enzyme exhibits trypsin-like endopeptidase activity, which preferentially cleaves peptide bonds at the carbonyl side of lysine or arginine residues. Tryptase is stored preformed in cytoplasmic granules, comprising 20–50% of the protein content of the mast cells. Stimulation of mast cells with antigens or other stimuli results in the release of active tryptase into the extracellular environment.

Emerging evidence suggests that tryptase plays a key role in the pathogenesis of asthma. Tryptase levels are elevated in the airways of asthmatic patients [3, 4]. *In vivo*, inhalation of tryptase into the airways results in bronchoconstriction and development of airway hyperresponsiveness through mast cell activation [5]. In addition, tryptase inhibitors have been shown to reduce antigen-induced airway responses *in vivo* [6].

The physiologic substrate(s) of tryptase have not been characterized fully. However, the cleavage of several peptides and proteins by tryptase suggests a contribution of the enzyme to asthma pathology. For example, tryptase hydrolyzes VIP†† [7] and calcitonin gene-related peptide [8], neuropeptides that provide endogenous bronchodilatory and vasodilatory activities, respectively. Tryptase also has kininogenase activity [9], which contributes to the generation of bradykinin [10]. In addition, tryptase may play a role in tissue degradation through cleavage of prostromelysin to its active form [11], which subsequently activates collagenase [12].

Cellular responses to tryptase also support the potential role of the protease in chronic inflammation and airway remodeling associated with asthma. Tryptase serves an amplification role in promoting allergic mediator release from mast cells [13] as well as potentiating bronchoconstriction [14]. Tryptase also may contribute to the late phase response through stimulation of leukocyte migration

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†† *Abbreviations*: VIP, vasoactive intestinal peptide; pNA, *p*-nitroanilide; P_{enh}, $Pause_{enhanced}$; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; and BABIM, bis(5-amidino-2-benzimidazolyl)methane.

into the airways [15]. In addition, tryptase exhibits mitogenic activity for fibroblasts [16], bronchial smooth muscle cells [17], and airway epithelial cells [18], which may contribute to pathologic changes of the asthmatic airway.

In this study, we characterized AMG-126737 as a potent and selective inhibitor of human mast cell tryptase. The efficacy of the compound was demonstrated in pharmacologic models of antigen-induced airway responses. These studies support the proposed role of tryptase in the pathology of asthma and suggest that AMG-126737 has potential therapeutic utility in this pulmonary disorder.

MATERIALS AND METHODS

Assays

Serine proteases were assayed using specific chromogenic peptide-pNA substrates in a 96-well microtiter plate format. Each protease was incubated with various concentrations of AMG-126737 for 5 min at 37° in specific assay buffer. The residual protease activity was measured following the addition of the respective substrate. The pNA product of proteolysis was quantified at 405 nm on a SpectraMAX 340 plate reader (Molecular Devices). Human lung tryptase (Cortex Biochem, Inc.) was assayed using tosyl-Gly-Pro-Arg-pNA in 50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.02% Triton X-100 [19]. Human plasma plasmin (Boehringer Mannheim) was assayed using tosyl-Gly-Pro-Lys-pNA (Sigma) in 100 mM Tris–HCl, pH 7.4, 100 mM NaCl, 0.05% Triton X-100 [20]. Bovine pancreatic trypsin (TPCK-treated) (Sigma) was assayed using *N*-α-benzoyl-L-Arg-pNA (Boehringer Mannheim) in 50 mM Tris–HCl, pH 8.2, 20 mM $CaCl_2$ [21]. Human plasma factor Xa (Calbiochem-Novabiochem International) was assayed using *N*-benzoyl-Ile-Glu-Gly-Arg-pNA (Pharmacia Hepar Inc.) in 50 mM Tris–HCl, pH 7.8, 200 mM NaCl, 0.05% BSA [20]. Human plasma (Calbiochem-Novabiochem International) and tissue kallikrein (prepared at Amgen) activities were assessed in 50 mM Tris–HCl, pH 7.8, 200 mM NaCl, 0.05% BSA using *H*-D-prolyl-Phe-Arg-pNA (Pharmacia Hepar Inc.) and DL-Val-Leu-Arg-pNA (Sigma), respectively [20]. Human plasma thrombin (Boehringer Mannheim) was assayed using *H*-D-Phe-Pip-Arg-pNA (Pharmacia Hepar Inc.) in 50 mM Tris–HCl, pH 8.3, 100 mM NaCl, 1% BSA [20]. Bovine pancreatic chymotrypsin (Boehringer Mannheim) was assayed using *N*-Suc-Ala-Ala-Pro-Phe-pNA (Sigma) in 100 mM Tris–HCl, pH 7.8, 10 mM $CaCl_2$ [22]. Human neutrophil cathepsin G (Calbiochem-Novabiochem International) was assayed using *N*-Suc-Ala-Ala-Pro-Phe-pNA (Sigma) in 625 mM Tris–HCl, pH 7.5, 2.5 mM $MgCl_2$, 0.125% Brij 35 [23]. Human neutrophil elastase (Calbiochem-Novabiochem International) was assayed using pyroGlu-Pro-Val-pNA (Pharmacia Hepar Inc.) in 100 mM Tris–HCl, pH 8.3, 0.96 M NaCl, 1% BSA [24]. The inhibition constants (K_i values) of AMG-126737 against each proteolytic enzyme were determined as previously described [25].

In addition, the inhibition of tryptase by AMG-126737 was evaluated using VIP (Sigma) as a substrate in 100 mM Tris–HCl (pH 8.0) with 1 μg/mL of heparin and 0.02% Triton X-100. VIP cleavage was assessed by reverse phase HPLC [26]. The K_i value was determined from measurements of fractional activity of tryptase at various drug concentrations.

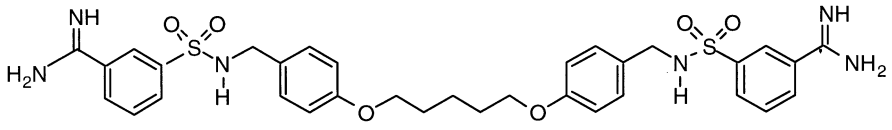
AMG-126737 also was evaluated for potential inhibition of interactions of allergic mediators with their specific receptors. Histamine binding to histamine H_1 receptor was assessed using bovine cerebellar membranes according to the method of Chang *et al.* [27]. Leukotriene D_4 binding to its receptor in guinea pig lung was assessed according to the method of Norman *et al.* [28]. Thromboxane A_2 binding to its receptor on human platelets was assessed according to the method of Hedberg *et al.* [29]. Receptor binding assays were conducted by NOVASCREEN.

Guinea Pig Airway Hyperresponsiveness

Male Hartley guinea pigs (Charles River Laboratories Inc.) were sensitized to ovalbumin by i.p. injection with a 0.5 mL solution of 10 μg ovalbumin and 10 mg aluminum hydroxide in PBS. Booster injections were administered at weeks 3 and 5 to ensure high titers of IgE and IgG_1 [30]. Seven to nine weeks after the initial injection, the animals were used to evaluate antigen-induced guinea pig airway responses.

To evaluate antigen-induced airway hyperresponsiveness in guinea pigs, a baseline histamine bronchoprovocation was conducted initially in unrestrained animals. Guinea pigs (450–600 g) were placed in a whole body plethysmograph (Buxco Electronics). The animals were exposed to 5-sec bursts of histamine aerosol generated by a DeVilbiss ultrasonic nebulizer. The bronchoconstrictor response was expressed as $Pause_{enhanced}$ (P_{enh}) [31]. P_{enh} can be conceptualized as the phase shift of the thoracic flow and the nasal flow curves. Increased phase shift correlates with increased respiratory system resistance. P_{enh} is calculated by the formula $P_{enh} = [(Te/RT) - 1][PEF/PIF]$, where Te is the total expiratory time, RT is relaxation time, PEF is peak expiratory flow, and PIF is peak inspiratory flow. The peak bronchoconstrictor response to rising histamine concentrations of 0, 25, 50, 100, and 200 mg/mL in PBS administered at 10-min intervals was determined. Three days after the histamine baseline determination, the guinea pigs were placed again in the whole body plethysmograph and exposed to ovalbumin for 30 min following a 3-sec aerosolized burst of 0.1% ovalbumin in PBS. Six hours after antigen exposure, the development of hyperresponsiveness was evaluated by repeating the histamine bronchoprovocation. Airway hyperresponsiveness was comparable when assessed 6 or 24 hr following antigen challenge. Comparisons between treatment groups were based on areas under the histamine dose–response curves. Administration of AMG-126737 or saline alone had no effect on baseline airway responsiveness to histamine (data not shown). At intratracheal doses up to 1 mg/kg, AMG-126737 had no inhibitory effect against antigen-induced immediate bronchoconstriction.

a.



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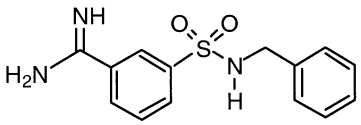

FIG. 1. Chemical structures of (a) AMG-126737 (1,5-bis-{4-[(3-carbamimidoyl-benzenesulfonylamino)-methyl]-phenoxy}-pentane) and (b) 3-benzylsulfamoyl benzamidine.

AMG-126737 was administered by intratracheal instillation in PBS (pH 7.2), 1 hr before antigen challenge. After anesthetizing a guinea pig with inhaled methoxyflurane, an endotracheal tube (18 gauge Teflon® sheath) was passed visually into the trachea with the aid of a fiberoptic light source. AMG-126737 (or PBS for control animals) was administered through the tube, followed by a bolus of air to facilitate dispersion. Alternatively, AMG-126737 was administered orally by gavage or by i.p. injection in 10% Trappsol (CTD Inc.) in water. No overt side-effects of AMG-126737 administration were observed.

Antigen-Induced Airway Responses in Sheep

AIRWAY MECHANICS. Adult ewes (median weight 30 kg) were instrumented as previously described [32]. Mean pulmonary flow resistance (R_L) was calculated from an analysis of 5–10 breaths by dividing the change in transpulmonary pressure by the change in flow at midtidal volume. Immediately after R_L determination, thoracic gas volume (V_{tg}) was measured in a constant volume body plethysmograph to calculate specific lung resistance (SR_L) by the equation $SR_L = R_L \cdot V_{tg}$.

A Raindrop jet nebulizer (Puritan-Bennett), operated at a flow rate of 6 L/min, was used to generate droplets with a mass median aerodynamic diameter of 3.6 ± 1.9 μm. Aerosol delivery was controlled using a dosimetry system [32], which was activated for 1 sec at the onset of the inspiratory cycle of a piston respirator (Harvard Apparatus Co.) Aerosols were delivered at a tidal volume of 500 mL and a respiratory rate of 20 breaths/min. AMG-126737 was delivered at physiologic pH in PBS.

Ascaris-sensitive sheep that exhibited both early and late phase bronchoconstriction were challenged with *Ascaris suum* extract (82,000 protein nitrogen Units/mL in PBS) (Greer Diagnostics) delivered as an aerosol at a rate of 20 breaths/min for 20 min. Changes in SR_L were monitored for 8 hr after antigen challenge.

AIRWAY HYPERRESPONSIVENESS. Baseline airway responsiveness was determined by measuring the SR_L immediately after saline inhalation and consecutive administration of 10 breaths of increasing concentrations of carbachol (0.25, 0.5, 1.0, 2.0, and 4.0%, w/v). Airway responsiveness was estimated by determining the cumulative carbachol breath units required to increase SR_L by 400% over the post-saline value (PC_{400}). One breath unit was defined as 1 breath of an aerosol containing 1% (w/v) carbachol [32]. Antigen-induced airway hyperresponsiveness was determined by repeating the carbachol dose–response study 24 hr after antigen challenge.

Statistical Analysis

Two-way ANOVA followed by the Newman–Keuls test was used to evaluate areas under the curve for histamine dose–responses in guinea pigs and SR_L in sheep. A paired *t*-test was used to evaluate changes in airway hyperresponsiveness in guinea pigs and sheep. The ED_{50} values were determined by linear regression analysis of dose–response data.

AMG-126737

AMG-126737 (1,5-bis-{4-[(3-carbamimidoyl-benzenesulfonylamino)-methyl]-phenoxy}-pentane) and its inactive analogue, 3-benzylsulfamoyl benzamidine (Fig. 1), were synthesized at a purity of > 95% as assessed by ^1H nuclear magnetic resonance spectroscopy. The structures of the compounds were confirmed by nuclear magnetic resonance and mass spectroscopy.

RESULTS

Specificity Profile of AMG-126737

The profile of serine protease inhibition by AMG-126737 (Fig. 1) is summarized in Table 1. Protease activities were assessed using chromogenic peptide pNA substrates. AMG-126737 was a potent inhibitor of human mast cell tryptase, with a K_i of 90 nM. In comparison, AMG-126737 inhibited tryptase-mediated cleavage of VIP with a K_i of 1.0 nM. AMG-126737 also exhibited selective inhibition of tryptase, with greater than 10- to 200-fold selectivity versus other trypsin-like serine proteases. In addition, AMG-126737 had no inhibitory effect against the chymotrypsin-like proteases (cathepsin G and chymotrypsin) or elastase.

TABLE 1. Protease inhibition profile of AMG-126737

Enzyme	Serine protease class	Substrate	K^i (nM)
Tryptase	Trypsin-like	tosyl-Gly-Pro-Arg-pNa	90
		Vasoactive intestinal peptide	1.0
Plasmin	Trypsin-like	tosyl-Gly-Pro-Lys-pNa	930
Trypsin	Trypsin-like	Nα-Benzoyl-L-Arg-pNA	2,500
Factor Xa	Trypsin-like	*N*-Benzoyl-Ile-Glu-Gly-Arg-pNA	6,400
Kallikrein (plasma)	Trypsin-like	*H*-D-Prolyl-Phe-Arg-pNA	18,000
Kallikrein (tissue)	Trypsin-like	DL-Val-Leu-Arg-pNA	No inhibition at 100 μM
Thrombin	Trypsin-like	*H*-D-Phe-Pip-Arg-pNA	No inhibition at 100 μM
Cathepsin G	Chymotrypsin-like	*N*-Suc-Ala-Ala-Pro-Phe-pNA	No inhibition at 100 μM
Chymotrypsin	Chymotrypsin-like	*N*-Suc-Ala-Ala-Pro-Phe-pNA	No inhibition at 100 μM
Elastase	Elastolytic	pyroGlu-Pro-Val-pNA	No inhibition at 100 μM

Antigen-Stimulated Airway Hyperresponsiveness in Guinea Pigs

AMG-126737 was evaluated for its effect on antigen-induced development of airway hyperresponsiveness in guinea pigs (Fig. 2). Six hours after antigen challenge, airway hyperresponsiveness, assessed as the area under the histamine dose–response curve, was increased 403% above control (N = 8, $P < 0.05$ vs baseline histamine response). Buffer alone had no effect on baseline airway responsiveness to histamine (data not shown). Intratracheal administration of AMG-126737 1 hr before antigen challenge provided a dose-dependent inhibitory effect against the development of hyperresponsiveness. Intratracheal instillation of AMG-126737 1 hr before antigen challenge inhibited the development of airway hyperreactivity, assessed as areas under the histamine dose–response curves, with an ED_{50} of 0.015 mg/kg. In contrast, 3-benzylsulfamoyl benzamidine (Fig. 1), an analogue of AMG 126737 that inhibited human lung mast cell tryptase with a K_i of only 100 μM, failed to exhibit inhibitory activity in the guinea pig airway hyperresponsiveness model.

Systemic activity of AMG-126737 also was examined in the guinea pig (Fig. 3). Treatment with a single 10 mg/kg dose of AMG-126737 by i.p. injection 1 hr before antigen challenge inhibited the development of airway hyperresponsiveness, assessed as area under the histamine dose–response curve (N = 5, $P < 0.003$). Activity of AMG-126737 also was observed following treatment with a single oral 10 mg/kg dose 1 hr prior to antigen challenge (N = 5, $P = 0.09$).

Antigen-Stimulated Bronchial Responses in Sheep

To confirm the pharmacologic activity of AMG-126737, the compound was evaluated for its effect against antigen-induced early and late bronchoconstriction in a sheep bronchoprovocation model. AMG-126737 (3 mg) was preadministered twice a day for 3 days and once 0.5 hr before antigen challenge. This regimen was selected based on the observation of Clark *et al.* [6] that prophylactic treatment with the tryptase inhibitor APC-366 resulted in a greater reduction of the early phase bronchoconstriction than was achieved following acute administration. AMG-126737 provided 34 and 95% inhibition of peak early- and late-phase bronchoconstriction, respectively (Fig. 4) (N = 2, $P < 0.1$ vs antigen-stimulated bronchoconstriction). Treatment with AMG-126737 reduced the areas under the curve for the early and late phase responses by 77% ($P = 0.15$ vs antigen-stimulated bronchoconstriction) and 92%

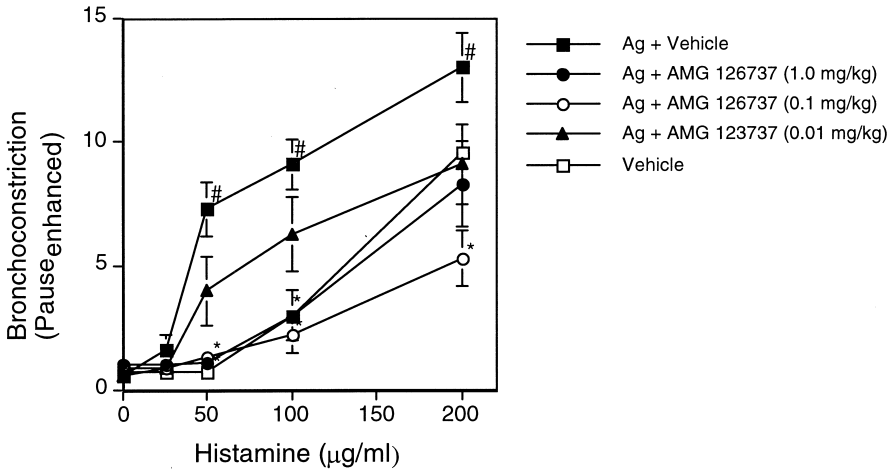

FIG. 2. Effect of AMG-126737 on antigen-induced airway hyperreactivity in guinea pigs. Hyperreactivity was determined as the shift of the dose-dependent bronchoconstriction (assessed as P_{enh}) in response to histamine, evaluated 6 hr after antigen challenge (means ± SEM, N = 8). Key: (#) $P < 0.05$, antigen-stimulated response vs baseline values; and (*) $P < 0.05$, drug treatment vs antigen-stimulated control response. Six hours after antigen challenge, airway hyperresponsiveness, assessed as the area under the histamine dose–response curve, was increased 403% above control ($P < 0.05$ vs baseline histamine response). Comparisons between treatment groups were based on areas under the histamine dose–response curves.

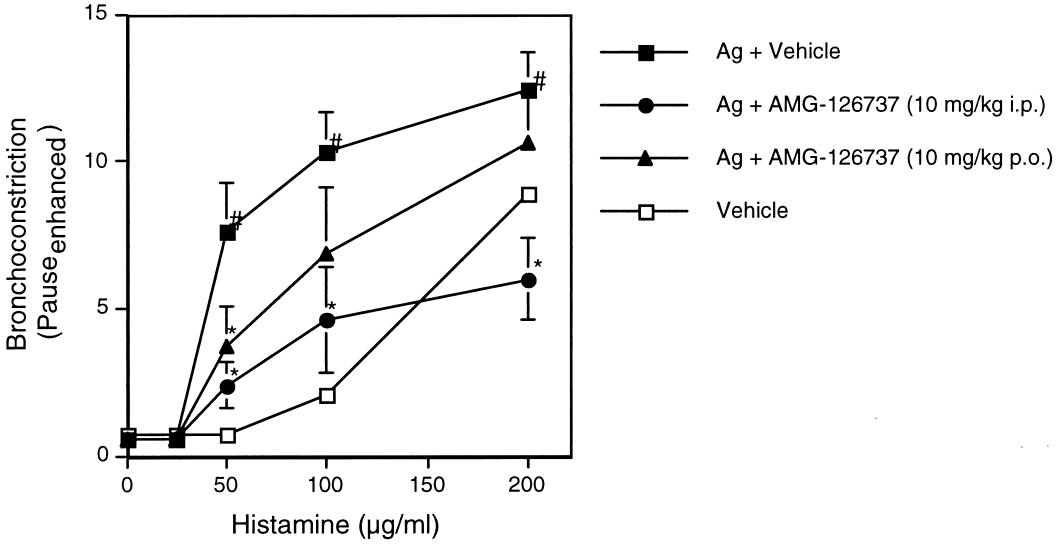

FIG. 3. Systemic activity of AMG-126737 against the development of allergen-induced airway hyperresponsiveness in guinea pigs. Hyperreactivity was determined as the shift of the dose-dependent bronchoconstriction (assessed as P_{enh}) in response to histamine, evaluated 6 hr after antigen challenge (means ± SEM, N = 8). Key: (#) $P < 0.05$, antigen-stimulated response vs baseline values; and (*) $P < 0.05$, effect of AMG-126737 vs antigen-stimulated response. Comparisons between treatment groups were based on areas under the histamine dose–response curves. Systemic administration of 10 mg/kg of AMG-126737 1 hr before antigen challenge inhibited the development of hyperresponsiveness (i.p. dose: N = 5, $P < 0.003$ vs antigen-stimulated control response; oral dose: N = 5, $P = 0.09$ vs antigen-stimulated control response).

($P = 0.10$ vs antigen-stimulated bronchoconstriction), respectively.

In these animals, AMG-126737 also was shown to prevent the development of airway hyperresponsiveness measured 24 hr following antigen challenge (Fig. 5). Airway hyperresponsiveness was assessed as the ratio of the carbachol dose required to induce a 400% change in airway resistance (PC_{400}) before and 24 hr after antigen challenge (mean ± range, N = 2). In buffer-treated animals, the ratio of pre- and post-antigen PC_{400}s was 0.51, indicating devel-

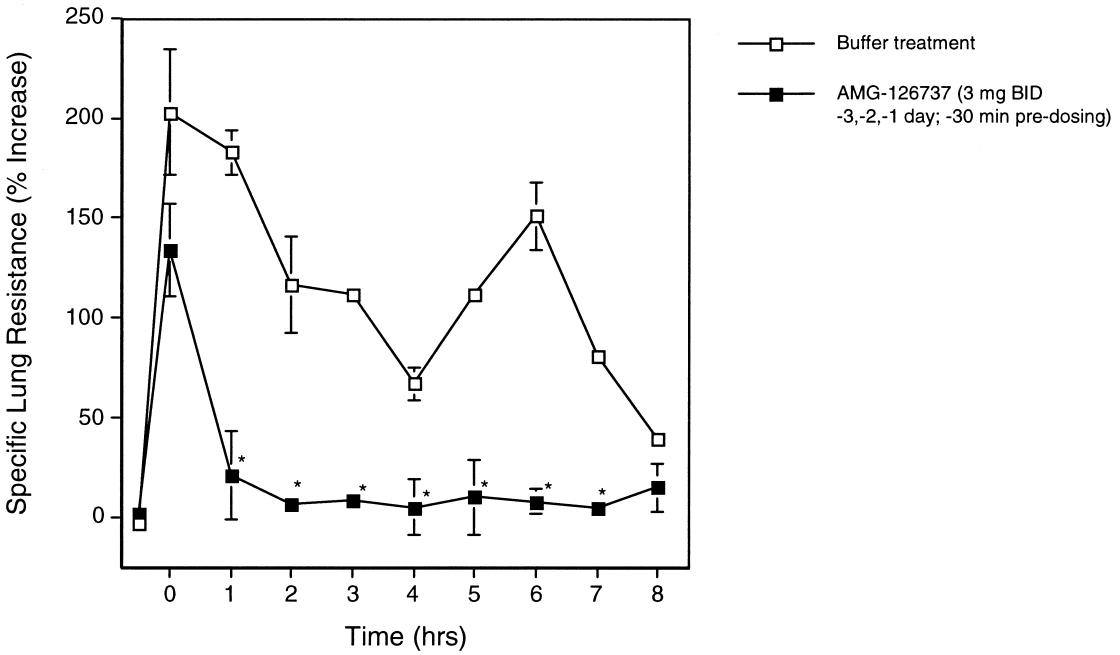

FIG. 4. Effect of AMG-126737 on antigen-stimulated bronchoconstriction in sheep. AMG-126737 was preadministered as a 3-mg aerosol dose twice a day (BID) for 3 days and 0.5 hr before antigen challenge. Early and late phase bronchoconstriction were assessed as the percent increase of specific lung resistance from a baseline of 0.98 ± 0.06 L · cm H_20/L/sec over an 8-hr period following antigen challenge (mean ± range, N = 2). Key: (*) $P < 0.1$, effect of AMG-126737 vs antigen-stimulated response. The areas under the curve for the early and late phase responses were reduced by 77% ($P = 0.15$) and 92% ($P = 0.10$), respectively, compared with antigen-stimulated control responses.

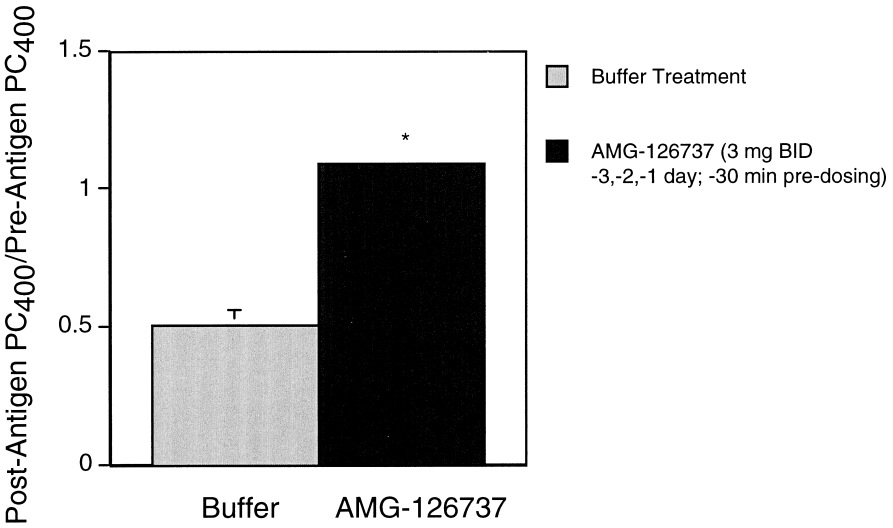

FIG. 5. Effect of AMG-126737 on antigen-stimulated airway hyperresponsiveness in sheep. Airway hyperresponsiveness was assessed as the ratio of the carbachol dose required to induce a 400% change in airway resistance (PC_{400}) before and 24 hr after antigen challenge (mean ± range, N = 2). Key: (*) $P <$ 0.05, effect of AMG-126737 vs control response.

opment of hyperresponsiveness. In contrast, in animals dosed with AMG-126737 prior to antigen challenge, as described above, the PC_{400} ratio was 1.1 ($P < 0.05$ effect of AMG-126737 vs control response). These results demonstrate that AMG-126737 prevented the development of airway hyperresponsiveness in antigen-challenged sheep.

DISCUSSION

Mast cell tryptase represents a novel therapeutic target for the treatment of asthma. The various cellular and tissue responses attributed to the enzyme make its exact mechanism of action unclear. However, the breadth of actions of tryptase suggests that the enzyme is a key upstream mediator of a cascade of biological responses that occur subsequent to mast cell activation. Pharmacologic data support the contribution of tryptase to allergen-induced airway responses [6].

In this report, we have demonstrated that AMG-126737 can provide effective therapy in preventing antigen-induced pathophysiologic airway responses, including early- and late-phase bronchoconstriction and development of airway hyperresponsiveness, in both guinea pig and sheep bronchoprovocation models. These results are consistent with the reported *in vivo* effects of the tryptase inhibitors BABIM and APC-366 [6].

AMG-126737 was designed to inhibit tryptase by occupying two S1 active sites of the tryptase homotetramer simultaneously [33, 34]. Although this novel, active site-bridging inhibitor can be classified as a bis-benzamidine, it is structurally dissimilar to the bis-benzamidine BABIM [35]. BABIM has been shown to inhibit trypsin through a zinc chelation mechanism mediated by its benzimidazole nitrogens in a 1,5 cyclic chelating arrangement [36]. Although untested, AMG-126737 is unlikely to possess the same ionophoric properties as BABIM, due to the lack of such chelating moieties.

AMG-126737 provides selective inhibitory activity against mast cell tryptase. The compound exhibited specificity for trypsin-like serine proteases, having greater than 10- to 200-fold selectivity versus plasmin, trypsin, factor Xa, and plasma kallikrein, while having no effect against tissue kallikrein or thrombin. More potent inhibition was observed when VIP, a natural target for tryptase hydrolysis in the human airway, was used as a substrate. The differences in K_i values for these substrates may result from the tetrameric nature of the enzyme. Crystallographic evidence suggests that the active centers of the monomers face a central pore allowing restricted access to macromolecular substrates. Binding of the inhibitor to two active sites of the tryptase homotetramer may further block proteolysis of larger protein substrates, such as VIP, by sterically hindering further enzyme–substrate interaction. For comparison with other reported tryptase inhibitors, APC-366 exhibits equipotent inhibition of tryptase, trypsin, and thrombin [6], whereas BABIM has been reported to have 10-fold selectivity for tryptase versus trypsin [35]. Selective inhibition of tryptase is critical to minimize potential adverse effects such as disruption of coagulation and fibrinolysis cascades.

The pharmacologic activity of AMG-126737 is associated with its tryptase inhibitory activity. 3-Benzylsulfamoyl benzamidine, a subunit of AMG-126737 with a greater than 3 log decrease in potency as a tryptase inhibitor, did not inhibit antigen-induced development of airway hyperresponsiveness in the guinea pig. In addition, at a 10 μM concentration, AMG-126737 had no antagonist activity against histamine H_1, leukotriene D_4, or thromboxane A_2 receptors (data not shown). However, these results do not preclude the possibility that AMG-126737 may possess other pharmacologic activities that may contribute to its *in vivo* efficacy.

It is important to consider whether inhibition of tryptase alone is sufficient to provide effective therapeutic intervention in asthma. Other serine proteases have been reported to contribute to airway responses associated with asthma. Leukocyte and tissue-derived serine proteases also are elevated in the airways of asthmatic patients [3, 4, 37]. In addition, such proteases, including cathepsin G [38, 39],

elastase [39–41], and tissue kallikrein [37], have been implicated in promoting physiologic responses associated with asthma as well as chronic airway remodeling associated with this disorder. It should be noted that tryptase inhibition can prevent increases of leukocyte protease levels indirectly by preventing leukocyte infiltration into the airways [6, 15]. Like tissue kallikrein, tryptase also has been shown to contribute to the generation of bronchoactive kinins [10]. However, the effect of tryptase inhibition on the protease tone of asthmatic airways remains to be characterized.

It is increasingly recognized that effective asthma therapy should prevent pathophysiologic airway responses such as recurrent bronchoconstriction and development of airway hyperresponsiveness as well as chronic pathologic changes of the asthmatic airway [42]. While its mechanistic roles have not been elucidated fully, tryptase appears to have an effector function in both asthma symptomatology and pathology. This and other reports [6] support the role of tryptase in antigen-induced effects on airway mechanics. The ability of tryptase to potentiate mast cell and leukocyte activation as well as smooth muscle responsiveness suggests that the enzyme is a key contributor to overall reactivity of the asthmatic airway. In addition, its mitogenic effects on fibroblasts and smooth muscle cells suggest that tryptase has a direct role in airway remodeling. Evaluation of the ability of tryptase inhibitors such as AMG-126737 to prevent chronic airway pathology is critical to fully assess the therapeutic potential of tryptase inhibition in asthma.

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