Antiasthmatic Activity of the Second-Generation Phosphodiesterase 4 (PDE4) Inhibitor SB 207499 (Ariflo) in the Guinea Pig

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Accepted for publication July 10, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

We evaluated the airway activity of the novel phosphodiesterase type 4 inhibitor SB 207499 [Ariflo; c-4-cyano-4-(3-cyclo- pentyloxy-4-methoxyphenyl-1-cyclohexane carboxylic acid], in the guinea pig. Ovalbumin (OA)-induced contractions of guinea pig isolated tracheal strips were inhibited by SB 207499 with an EC50 of 1 μM but had little or no effect on exogenous agonist-induced contraction, which suggests that its effect on OA-induced contraction in vitro is primarily due to inhibition of mediator release from mast cells. In anesthetized guinea pigs, SB 207499 inhibited OA-induced bronchoconstriction with i.v. and p.o. ID50 values of 1.7 and 17 mg/kg, respectively. At 1, 3 and 6 hr after SB 207499 (30 mg/kg p.o.), OA-induced bronchospasm was inhibited by 92%, 70% and 58%, respectively, corresponding to elevated plasma concentrations of 1.62 ± 0.19, 1.65 ± 0.29 and 0.93 ± 0.24 μg/ml, respectively, of SB 207499. SB 207499 also inhibited house dust mite-induced bronchoconstriction (ID50 = 0.9 mg/kg i.v. and 8.9 mg/kg p.o.). In contrast to its lack of bronchorelaxant activity in vitro, SB 207499 inhibited bronchospasm induced by i.v. leukotriene D4 (LTD4) [ID50 = 3 mg/kg i.v.]. The bronchorelaxant effect of i.v.-administered SB 207499 was at least additive with that of salbutamol in reversing infused histamine-enhanced airway tone, but it did not alter base line or enhance salbutamol-induced cardiovascular effects. In conscious guinea pigs, SB 207499 (10 or 30 mg/kg p.o.) 1 hr before antigen or LTD4 challenge, markedly reduced bronchospasm and subsequent eosinophil influx as measured by bronchial lavage 24 hr after provocation. SB 207499 administered after OA or LTD4 challenge also reduced airway eosinophilia measured at 24 hr after OA challenge or 96 hr after LTD4 challenge. These results, coupled with the broad anti-inflammatory activity of SB 207499 previously described (Barnette et al., 1998), suggest that SB 207499 will be useful in the treatment of asthma and other inflammatory disorders.

Increases in cellular concentration of cAMP mediate relaxation of airway smooth muscle and inhibit chemotaxis, cytotoxicity and activation of inflammatory cells (Torphy and Hay, 1990; Giembycz and Raeburn, 1991; Torphy and Undem, 1991). Inactivation of this second messenger is catalyzed by a family of PDE isozymes (Beavo and Reifsnyder, 1990). Among the forms of PDE is the cAMP-specific isozyme PDE4, which is a major component of the catabolic system for cAMP in airway smooth muscle and in inflammatory and immunocompetent cells (for review, see Torphy, 1998). This information underlies the considerable interest in PDE4 as a target for novel antiasthmatic drugs (Torphy, 1998). Indeed, rolipram, the archetypical selective inhibitor of PDE4 (Schwabe et al., 1976), displays a broad range of anti-inflammatory (Dent et al., 1991; Torphy and Undem, 1991) and bronchodilatory activity (Harris et al., 1989).

In actively sensitized guinea pigs, antigen provocation elicited bronchoconstriction and pulmonary eosinophil influx (Patterson and Kelly, 1974; Dunn et al., 1988). Histamine, prostaglandins, leukotrienes and cytokines are inflammatory cell mediators that are implicated in these events (Barnes et al., 1988). Rolipram produces marked inhibitory effects when employed in antigen-driven animal models that share a number of pathophysiologic sequelae found in human asthma (Underwood et al., 1993, 1994; Howell et al., 1993; Turner et al., 1994). Despite the substantial therapeutic activity of rolipram and other first-generation PDE4 inhibitors, their clinical utility is limited by several side effects, including nausea, emesis and gastric acid secretion (Bertolino et al., 1988; Hebenstreit et al., 1989; O’Connolly et al., 1988; Heaslip and Evans, 1995; Barnette et al., 1996). It is this background of potential therapeutic advantage that has inspired efforts to discover a second generation of selective PDE4 inhibitors with improved clinical profiles.

ABBREVIATIONS: PDE4, phosphodiesterase type 4; OA, ovalbumin; HDM, house dust mite; LTD4, leukotriene D4; SB 207499 (Ariflo), c-4-cyano-4-3-cyclopentyloxy-4-methoxyphenyl-1-cyclohexane carboxylic acid; BAL, bronchoalveolar lavage.
inhibitors with an improved side effect profile (Hughes et al., 1996; Barnette et al., 1998; Souness, 1997; Torphy, 1998).

SB 207499 is a second-generation inhibitor of PDE4 designed specifically to retain the therapeutic activity of first-generation compounds but to offer an improved side effect profile (Torphy et al., 1997; Barnette et al., 1998; Griswold et al., 1998; Christensen et al., 1998). The objective of this study was to provide an in-depth evaluation of the pulmonary pharmacology of SB 207499. The results indicate that SB 207499 exerts a broad spectrum of anti-inflammatory and bronchodilatory activities that highlight the potential anti-asthmatic activity of this second-generation PDE4 inhibitor.

**Materials and Methods**

**Sensitization Procedure**

OA. Male Hartley guinea pigs (200–250 g) were sensitized by i.m. injections of 0.35 ml of a 5% (w/v) OA/saline solution into each thigh (0.7 ml total) on days 1 and 4. Guinea pigs were available for use after day 25.

HDM. Male Hartley guinea pigs (200–250 g) were sensitized by injections of HDM (equal-parts mixture of D. pteronyssinus and D. farinae) in 50% glycerin (5000 antigenic units (Au)/ml) (Greer Laboratories, Lenoir, NC) diluted 1:5 in 0.9% saline (1000 Au/ml). Aluminum hydroxide was added to diluted antigen at a concentration of 10 mg/ml. Animals were sensitized with 0.3 ml of HDM/saline solution plus alum, administered s.c. on days 1, 3 and 5. Guinea pigs were available for use after day 38.

**Static Tissue Bath Experiments**

OA-sensitized guinea pigs (500–800) were sacrificed by cervical dislocation, and the tracheae were removed and cut into two cartilage-wide strips. Each individual tracheal preparation was suspended in a 10–ml water-jacketed tissue bath containing Krebs buffer of the following composition (mM): NaCl 118, KCl 4.7, CaCl2 2.5, MgCl2 0.5, NaH2PO4 1.0, NaHCO3 21.4, and dextrose 2.0. The buffer was maintained at 37°C and continuously aerated with 95% O2/5% CO2. The opposite ends of the tracheae were tied to a glass rod tissue holder and a Grass model FT03C force-displacement transducer (Grass Instrument Co., Quincy, MA) for the recording of isometric tension on a Grass polygraph and then placed under 8 g of tension. After a 1-hr equilibration period (during which time the tissues were washed every 15 min with fresh Krebs buffer), each tracheal strip was pretreated for 30 min with either dimethyl sulfoxide vehicle or SB 207499 (0.1, 1 or 10 μM). Concentration-response curves were constructed by cumulative increases in the bath concentration of OA, with each concentration remaining in contact with the tissue until the response reached a plateau before addition of the subsequent agonist concentration (VanRossum, 1963). Only one concentration-response curve was generated with each tissue. At the end of the study, 10 μM carbachol was added to obtain a maximum contraction, and all data were expressed as a percent of the carbachol-induced maximum response.

**Spasmogen Studies in Anesthetized Guinea Pigs**

Guinea pigs (600–800 g) were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and the jugular vein, carotid artery and trachea were cannulated for i.v. drug administration, blood pressure monitoring and ventilation, respectively (Underwood et al., 1992, 1993, 1996b). Bilateral vagotomies were performed to minimize the influence of neural reflexes. The animals were paralyzed with pancuronium bromide (0.1 mg/kg i.v.) and ventilated at 45 breaths/min. Airway pressure changes were measured via a side arm of the tracheal cannula with a Druck PDCR 102L, 70-mBar transducer (Druck Incorporated, New Raifeld, CT). The ventilatory stroke volume of a Harvard rodent respirator (model 683) was set to produce a side-arm pressure of 8 cm of H2O (~5 cc room air). Blood pressure was measured with a Kobe Model CDXIII disposable pressure transducer (Kent Scientific, Litchfield, CT). Pressures were recorded on a thermal chart recorder (Model WR 3300, Western Graphitec, Irvine, CA).

**Inhibition of Antigen-Induced Bronchoconstriction**

OA-induced bronchoconstriction was elicited by i.v. administration of 0.1 mg/kg OA. At the peak of the initial response to OA, a subsequent dose of 0.2 mg/kg OA (0.3 mg/kg, cumulative) was added. At the peak of the antigen-induced response, 1 cc/kg of a saturated KCl solution was administered. Responses to OA were expressed as a percent of the maximum bronchoconstriction induced by KCl. SB 207499 or vehicle, 90% polyethylene glycol (PEG) 400 in saline, was administered i.v. 10 min before or intragastrically (i.g.) 1 hr before OA challenge. Inhibitory activity by SB 207499 was expressed as percent inhibition comparing responses with that of a set of vehicle-treated animals.

For HDM-induced bronchoconstriction, SB 207499 (0.1–3 mg/kg i.v.) was administered 10 min before challenge by 1 ml/kg of a 3500 Au/ml solution of HDM antigen solution.

**Time Course Study**

SB 207499 (30 mg/kg) or vehicle (90% PEG 400 in water) was administered i.g. 1, 3 or 6 hr before OA challenge. Blood samples (1 ml) were drawn immediately before OA challenge, and plasma was isolated and frozen for drug analysis. Guinea pigs were challenged as previously described with OA (0.3 mg/kg), and percent inhibition of the control bronchoconstrictor response to OA was determined. Plasma concentrations of SB 207499 were determined via a liquid-liquid (0.1 N acetic acid/hexane:methyl-t-butyl ether (1:1) extraction procedure using a validated gas chromatographic method employing a thermionic detector.

**Inhibition of LTD4-Induced Bronchoconstriction**

SB 207499 (1–10 mg/kg i.v.) or vehicle was administered to anesthetized guinea pigs that had been instrumented as described previously. Ten minutes later, LTD4 (0.3 μg/kg i.v.) was administered and airway insufflation pressure increases were measured. Results were calculated as percent inhibition by SB 207499 when compared with vehicle-treated controls. Only one dose of SB 207499 was given to each animal.

**Bronchodilation in the Anesthetized Guinea Pig**

Animals were anesthetized and instrumented as described above. A hyperinflation (2× tidal volume) was performed, and ventilatory volume was adjusted to obtain an inflation pressure of 8 cm H2O. After a 5-min stabilization period, a continuous infusion (Harvard Apparatus Infusion Pump Model 22) of histamine diHCl (100 μg/ml) was initiated. The infusion rate (6–12 ml/hr) was adjusted in order to elevate inflation pressure and maintain it at 20 to 25 cm H2O (2–3-fold above basal). After a steady-state bronchospasm was achieved (10–20 min after the start of the histamine infusion), SB 207499 or vehicle was administered as i.v. bolus injections of ascending doses. Results were expressed as percent reduction in histamine-induced tone. In another group of animals in which a stable histamine-induced tone (2- to 3-fold above basal) was achieved, vehicle or SB 207499 (0.3 or 3 mg/kg i.v.) was administered, followed 10 min later with increasing doses of salbutamol (0.01–10 μg/kg i.v.). Two minutes were allowed between doses of salbutamol so that a stable airway pressure could be obtained. Results were expressed as percent reductions of histamine-induced airway tone.

**Effect of SB 207499 on Basal and Salbutamol-Induced Vasoactivity and HR**

In a separate group of animals, vehicle or SB 207499 (3 or 10 mg/kg i.v.) was administered 10 min before the measurement of
Conscious-Animal Body Plethysmography

A. Antigen studies. Male Hartley guinea pigs (550–750 g), actively sensitized to OA, were pretreated with chlorpheniramine (0.1 mg/kg s.c.) 15 min before antigen challenge and placed into a double-flow body plethysmograph (Penn-Century, Philadelphia, PA) consisting of a nasal (head) chamber and a thoracic (body) chamber, each equipped with a pneumotachograph. The plethysmograph was connected to a Noninvasive Respiratory Analyzer (Buxco Electronics, Sharon, CN) via a Validyne differential pressure transducer (+2 cm) that calculated specific airway conductance (sGaw). After a 10-min stabilization period, an aerosol of OA (1% in normal saline) was generated by an ultrasonic nebulizer (Pulmosonic, DeVilbiss Corporation, Somerset, PA) and delivered for 10 sec at a rate of 250 ml/min via a nosecone built into the plethysmograph.

Results were calculated as percent change in sGaw from base-line readings taken just before spasmogen challenge. OA-induced changes in sGaw are reported every minute for 6 min and then every 2 min until 10 min after challenge. SB 207499 (10 or 30 mg/kg or vehicle (90% PEG 400 in water) was administered i.g. through a size 8 French feeding tube 1 hr before antigen challenge.

BALs were performed 24 hr after OA exposure. Guinea pigs were killed by cervical dislocation and exsanguinated. The lungs were lavaged with 50 ml of Dulbecco’s PBS (5 x 10 cc), which was aspirated after a gentle chest massage. The BAL fluid was spun down, and the pellet was resuspended in 0.25% NaCl to lyse residual erythrocytes. After centrifugation, the pellet was resuspended again in 0.9% NaCl. After a total cell count, slides were prepared and stained. The cells were differentiated as eosinophils, neutrophils and mononuclear cells by counting a minimum of 200 cells and expressing the results as a percentages of the total number of cells as well as actual numbers of each type.

Administration of SB 207499 after antigen challenge: In a separate group of animals, SB 207499 (30 mg/kg p.o.) was administered 2 hr after antigen provocation. With the exception of the timing of the treatment with SB 207499, the protocol (i.e., chlorpheniramine pre-treatment, bronchoconstriction measurement, BAL 24 hr after antigen exposure) was the same as described previously.

B. LTD4 studies. Male Hartley guinea pigs were treated much as in the antigen studies, except that the animals were neither sensitized to OA nor pretreated with chlorpheniramine, and an aerosol of LTD4, 10 μg/ml, was administered to guinea pigs for 1 min. Bronchoconstriction and inflammatory cell influx were measured using the aforementioned protocol in the antigen experiments. The LTD4-induced peak reduction in sGaw is detected at different time-points after challenge in different animals, but it generally occurs 2 to 4 min after inhalation of LTD4. Therefore, the overall LTD4-induced bronchoconstriction is best represented by an area-under-the-curve analysis of the percent reduction in sGaw.

Effect of subchronic SB 207499 posttreatment on LTD4-induced persistent eosinophilia: Because we have previously shown that a single exposure to LTD4 results in a persistent airway eosinophilia that peaks at 4 days and remains elevated for 2 to 4 weeks (Underwood et al., 1996b), vehicle or SB 207499 (10 or 30 mg/kg p.o.) was administered 2 hr and 6 hr after aerosol LTD4 exposure (10 μg/ml for 1 min) and b.i.d. thereafter for 4 days. Eosinophilia was measured as outlined above.

Materials
SB 207499 was synthesized in the laboratory of Dr. Siegfried Christensen, and LTD4 and salbutamol were synthesized by the Department of Medicinal Chemistry at SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Histamine dihydrochloride, carbachol (carbamylcholine chloride), chlorpheniramine maleate and OA (chicken egg, grade V) were purchased from Sigma Chemical Co. (St. Louis, MO). Pancuronium bromide (Pavulon) was purchased from Organon Inc. (West Orange, NJ). HDM (D. pteronyssinus and D. farinae in glycerin) was purchased from Greer Laboratories (Leenoir, NC).

Results

Static tissue bath experiments. In tracheal strips isolated from OA-sensitized guinea pigs, exogenous OA produces concentration-related contractions of approximately 70% of the maximum contraction elicited by 10 μM carbachol (fig. 1). SB 207499 (0.1–10 μM) inhibited antigen-induced contractions in a concentration-dependent manner with an EC50 of 0.1 to 1.0 μM, depending on the concentration of OA used. SB 207499 (10 mM) did not significantly alter the concentration-related contraction induced by exogenous carbachol (data not shown).

Antigen-induced bronchoconstriction in anesthetized guinea pigs. As previously shown (Underwood et al., 1996a) and confirmed in the present study, OA (0.3 mg/kg i.v.) produces a bronchoconstriction in anesthetized guinea pigs characterized by a pressure increase of approximately 70 cm H2O, which represents about 90% of the KCl-induced maximum airway response. Pretreatment of guinea pigs with i.v. (10 min before) or p.o. (1 hr before) SB 207499 inhibited OA-induced bronchoconstriction with ID50 values of 1.7 and 17 mg/kg, respectively (fig. 2).

SB 207499 time course study. In this study, pretreatment of guinea pigs with SB 207499 (30 mg/kg p.o.) 1, 3 or 6 hr before antigen challenge resulted in 92%, 70% and 58% inhibition, respectively, of OA-induced bronchoconstriction when compared with appropriate time-matched, vehicle-treated control animals (fig. 3). The inhibitory activity at 1, 3 and 6 hr corresponded to SB 207499 plasma concentrations of 1.62 ± 0.19 μg/ml, 1.65 ± 0.29 μg/ml and 0.93 ± 0.24 μg/ml, respectively (n = 4, fig. 3).

HDM study. Additional experiments were conducted to assess the effect of SB 207499 on airway responses to a clinically relevant antigen, HDM. HDM (3500 Au/kg) produced a bronchoconstriction characterized by an increase in airway insufflation pressure of 73 ± 2 cm H2O, approximately the same magnitude as that described with OA. Pretreatment with SB 207499 inhibited the HDM-induced bron-
choconstriction with i.v. and p.o. ID$_{50}$ values of 0.9 and 8.9 mg/kg, respectively (data not shown).

**Inhibition of LTD$_4$-induced bronchoconstriction.** Administration of LTD$_4$ (0.3 µg/kg i.v.) produces a peak in airway insufflation pressure of approximately 20 to 23 cm H$_2$O, which constitutes approximately 30% of the maximum airway response to a saturated solution of KCl (Underwood et al., 1993; Bochnowicz and Underwood, 1995). SB 207499 (1–10 mg/kg i.v.), administered 10 min before exogenously administered LTD$_4$, produced a dose-dependent inhibition of the LTD$_4$-induced bronchospasm with an ID$_{50}$ of approximately 3 mg/kg i.v. (data not shown). The maximum inhibition attained at the highest dose tested, 10 mg/kg i.v., was 68 ± 3%.

**Reversal of histamine-induced airway tone by SB 207499 and salbutamol.** In a group of animals in which airway tone was raised 200% to 300% by constant i.v. infusion of histamine, additive administration of SB 207499 (0.03–3 mg/kg i.v.) produced a dose-dependent reduction in tone that plateaued at about 45% inhibition at 0.3 mg/kg (0.43 mg/kg, cumulative), i.v. (fig. 4A). Administration of a total volume of vehicle corresponding to the entire dose range of SB 207499 (5 × 1 ml) produced an 18% decrease of the histamine-induced airway tone (fig. 4A).

In a separate experiment, the beta adrenoceptor agonist salbutamol (0.01–10 µg/kg i.v.) produced a dose-dependent relaxation of histamine-induced airway tone in vehicle-treated animals. Pretreatment with SB 207499 (0.3 or 1 mg/kg i.v.) produced a 9% and 45% relaxation, respectively, in histamine-induced tone. The bronchorelaxant effect of SB 207499 was at least additive to that of salbutamol. However, the maximum reduction in histamine-induced tone with com-
bined salbutamol and SB 207499 (55 ± 2%) was not significantly different from SB 207499 (1 mg/kg i.v., 45 ± 2%) or salbutamol (10 μg/kg, 52 ± 2%) alone (fig. 4B).

**Cardiovascular effects of SB 207499 and salbutamol.** In a separate group of anesthetized guinea pigs, the effect of SB 207499 on the vasodepressor effects of salbutamol was assessed (fig. 5A; table 1). SB 207499 (3 or 10 mg/kg i.v.) neither lowered base-line mean arterial pressure (table 1) nor significantly altered the systemic arterial depressor effects of salbutamol (0.01–10 μg/kg i.v.; fig. 5A). At the maximum dose tested, 10 μg/kg i.v., salbutamol reduced mean arterial pressure by greater than 60% (fig. 5A). In the same animals, salbutamol dose-dependently increased HR to a maximum of 35% above the resting rate (fig. 5B). Neither the resting HR nor the changes in HR elicited by any dose of salbutamol were altered by pretreatment with SB 207499 (3 or 10 mg/kg i.v.; fig. 5B).

**OA-induced bronchoconstriction and airway eosinophilia in conscious guinea pigs.** In conscious, OA-sensitized guinea pigs pretreated with chlorpheniramine (0.1 mg/kg s.c.), a 10-sec aerosol administration of OA (1%) produced a bronchoconstriction characterized by a 51.8 ± 4.1% decrease from base-line sGaw over 10 min (n = 11; fig. 6A). When administered p.o. 1 hr before antigen challenge, SB 207499 (10 or 30 mg/kg) dose-dependently inhibited (15% and 42% reduction, respectively, compared with vehicle-treated animals; P < .05, ANOVA) the OA-induced bronchoconstriction (maximum sGaw decrease = 44.2 ± 3.2% and 29.8 ± 4.6%, respectively; n = 10; fig. 6A). The base-line sGaw in animals treated with SB 207499 (10 or 30 mg/kg, p.o. = –0.17 ± 0.02 sec⁻¹ (cm H₂O)⁻¹ and –0.16 ± 0.03 sec⁻¹ (cm H₂O)⁻¹, respectively) was not significantly different from that in vehicle-treated control animals (–0.22 ± 0.03 sec⁻¹ (cm H₂O)⁻¹; P > .05, ANOVA; n = 10–11). Qualitatively, neither dose of SB 207499 produced any noticeable behavioral changes in guinea pigs, a result that contrasts markedly with the enhanced excitability exhibited in animals after rolipram treatment at a 10-fold lower dose (Underwood et al., 1993; Underwood et al., 1996a). When the same animals were subjected to BAL 24 hr after antigen challenge, the total inflammatory cell number, and especially the eosinophil count, expressed both as a fraction of total inflammatory cells recovered (40 ± 4%) and as actual number of eosinophils recovered (3.5 ± 0.6 × 10⁶ cells), were significantly increased compared with BAL eosinophil concentrations in animals that were not exposed to antigen (6.0 ± 0.5%; 0.44 ± 0.06 × 10⁶ cells; not shown) (fig. 6B). Pretreatment with SB 207499 (10 or 30 mg/kg p.o.) inhibited the OA-induced eosinophil infiltration (fig. 6B). Indeed, when assessment was made as absolute numbers of cells SB 207499 virtually abolished antigen-induced eosinophil influx (fig. 6B).

In addition to studying the effect of pretreatment with SB 207499 on antigen-induced eosinophilia, we sought to analyze the effects of this selective PDE4 inhibitor when animals are treated after antigen challenge. When SB 207499 (30 mg/kg p.o.) was administered 2 hr after antigen challenge, there was a significant reduction (>50%) in the OA-induced eosinophilia, expressed as both the actual number and the percent of total cells recovered in the BAL fluid (P < .05, Student’s unpaired t test, two-tailed; fig. 7).

**LTD₄-induced bronchoconstriction and airway eosinophilia.** Aerosol administration of LTD₄ produces a profound bronchoconstriction and a persistent airway eosinophilia in the guinea pig (Underwood et al., 1996b). In this study, aerosol LTD₄ (10 μg/ml for 1 min) reduced sGaw by 88 ± 0.6% (mean ± S.E.M. of maximum of each animal; n = 8; not shown). The LTD₄-induced peak reduction in sGaw was detected at different time-points in different animals, but it generally occurred 2 to 4 min after inhalation of LTD₄. Therefore, the overall LTD₄-induced bronchoconstriction was best represented by an area-under-the-curve (AUC) analysis of the percent reduction in sGaw. In vehicle-treated guinea pigs, the bronchoconstriction-related AUC was 791.3 ± 15.4% reduction in sGaw over 10 min (n = 8). LTD₄-induced bronchoconstriction was significantly inhibited in animals pretreated with SB 207499 (10 or 30 mg/kg p.o., 1 hr before challenge), which resulted in AUC measurements of 676.0 ± 49.3% (n = 5; P = .02) and 637.5 ± 44.7% (n = 5; P = .003), respectively (ANOVA, Fisher’s PLSD). Although there was a significant reduction of the overall LTD₄-induced bronchoconstriction by p.o. pretreatment with 10 or 30 mg/kg SB 207499, there was no significant inhibition of the peak LTD₄-induced airway response (not shown). In an analysis of the inflammatory cell influx into the lung as assessed by BAL, both doses of SB 207499 substantially reduced (60–80% vs. vehicle-treated animals) LTD₄-induced eosinophilia (fig. 8A; n = 5 for each dose; P < .05, ANOVA, Fisher’s PLSD).

In another series of experiments assessing the effects of SB 207499 on the persistent eosinophilia induced by inhaled
LTD₄, SB 207499 (10 or 30 mg/kg p.o., b.i.d.) was administered beginning 2 hr after LTD₄ challenge for 4 days. In this protocol, the 30 mg/kg dose, but not the 10 mg/kg dose, significantly reduced the resulting persistent eosinophilia measured at 96 hr after LTD₄ challenge (fig. 8B, \( n = 7-8 \), \( P < .05 \), ANOVA, Fisher’s PLSD).

**Discussion**

The objective of this study was to establish a comprehensive profile of the airway pharmacology of SB 207499, a second-generation PDE4 inhibitor undergoing clinical evaluation for the treatment of asthma. To this end, the key actions of SB 207499 in anesthetized or conscious guinea pigs include the following: 1) inhibition of antigen-induced bronchoconstriction and eosinophil influx into the airway; 2) inhibition of persistent LTD₄-induced airway eosinophilia; 3) prevention or reversal of histamine- or LTD₄-induced bronchoconstriction; 4) prolonged duration of action correlating with elevated drug plasma concentrations; 5) lack of hemodynamic or cardiac effects, either alone or in combination with salbutamol. These major findings provide a strong rationale for the potential antiasthmatic activity of SB 207499.

In addition to the major findings described above, SB 207499 produced a concentration-dependent inhibition of OA-induced contractions of guinea pig isolated trachea. In contrast, SB 207499 had little or no effect on contractions of isolated trachea induced by carbachol. These results are consistent with those obtained previously with rolipram (Underwood et al., 1993) and suggest that in vitro, the ability of PDE4 inhibitors to suppress antigen-induced contraction of isolated airway is due to inhibition of mast cell degranulation rather than to direct bronchodilatation.

Although in vitro data do not support a substantial bronchodilator effect with the PDE4 inhibitors, SB 207499 both inhibited and reversed LTD₄- or histamine-induced bronchoconstriction in vivo. The in vivo bronchodilatation may be explained by the ability of SB 207499, like other PDE4 inhibi-

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Blood Pressure (mmHg)</th>
<th>HR (beats per minute)</th>
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<tr>
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<td>Diastolic</td>
<td>Systolic</td>
</tr>
<tr>
<td>Base line</td>
<td>51.3 ± 4.6</td>
<td>74.1 ± 7.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>56.9 ± 4.3</td>
<td>84.4 ± 8.3</td>
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<tr>
<td>Base line</td>
<td>56.8 ± 6.8</td>
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<tr>
<td>SB 207499 3.0 mg/kg i.v.</td>
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<td>91.3 ± 0.6</td>
</tr>
<tr>
<td>SB 207499 10 mg/kg i.v.</td>
<td>60.6 ± 2.8</td>
<td>89.7 ± 5.0</td>
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Results are given as the mean ± S.E.M.; \( n = 4 \).

**Fig. 6.** Effects of SB 207499 on aerosol OA-induced bronchoconstriction (panel A) and airway eosinophil influx (panel B). SB 207499 (10 or 30 mg/kg p.o.) was administered 1 hr before OA (1% for 10 sec) challenge. Eosinophils were measured in BAL fluids 24 hr after challenge. Eosinophils are expressed either as a percentage of total cells (% eosinophils) or as absolute numbers (eosinophil #). Results are given as mean ± S.E.M.; \( n = 4 \) to 11. *P < .05; ANOVA, Fisher’s PLSD.

**Fig. 7.** Effects of post-treatment with SB 207499 on aerosol OA (1% for 10 sec)-induced airway eosinophil influx. SB 207499 (30 mg/kg p.o.) was administered 2 hr after OA (1% for 10 sec) challenge. Eosinophils were measured in BAL fluids 24 hr after challenge. Eosinophils are expressed either as a percentage of total cells (% eosinophils) or as absolute numbers (eosinophil #). Results are given as mean ± S.E.M.; *P < .05, Student’s unpaired t test (two-tailed); \( n = 7 \) to 8.
Inhibition of antigen-induced bronchoconstriction in anesthetized guinea pigs is a standard model used to provide an initial assessment of the in vivo activity of PDE4 inhibitors. We compared the activity of SB 207499 against two different antigens, OA and the more clinically relevant HDM. SB 207499 inhibited antigen-induced bronchoconstriction with i.v. ID$_{50}$ = 1.7 mg/kg and p.o. ID$_{50}$ = 17 mg/kg. Notably, SB 207499 had a prolonged duration of action, substantially inhibiting OA-induced bronchoconstriction for at least 6 hr after its p.o. administration. This long duration of action was paralleled by elevated plasma concentrations of SB 207499.

In the HDM-induced bronchoconstriction model, SB 207499 administered b.i.d., 2 hr and 6 hr post-treatment protocol, SB 207499 (10 or 30 mg/kg p.o.) was administered 1 hr before LTD$_4$ (10 µg/ml for 1 min) challenge and then b.i.d. 6 hr apart for 4 days. Eosinophils were measured in BAL fluids 24 hr after challenge (panel A). In the pretreatment protocol, SB 207499 (10 or 30 mg/kg p.o.) was administered 1 hr before LTD$_4$ (10 µg/ml for 1 min) challenge and then b.i.d. 6 hr apart for 4 days. Eosinophils were measured in BAL fluids 24 hr after challenge. Eosinophils are expressed either as a percentage of total cells (% eosinophils) or as absolute numbers (eosinophil #) (Underwood et al., 1996a).

In vitro studies with SB 207499 and other PDE4 inhibitors indeed suggest multiple possibilities, including inhibition of antigen-driven IL-5 production (Barnette et al., 1998), cytokine-induced adhesion to endothelial cells (Torphy et al., 1994) and chemotaxis (Cohan et al., 1996; Kaneko et al., 1995). The lack of direct cardiovascular effects of SB 207499 and the finding that SB 207499 did not exacerbate the cardiovascular effects of salbutamol has two important connotations: PDE4 is probably not the most important PDE isozyme in cAMP-mediated cardiotonic and capacitance vessel relaxation, and the profile of cardiovascular side effects of SB 207499, employed either alone or in combination with a beta adrenoceptor activating bronchodilator, is attractive. In contrast to the failure of SB 207499 to exacerbate the cardiovascular effects of salbutamol, the PDE4 inhibitor exhibited bronchodilatory effects that were at least additive with that of the beta agonist.

In summary, SB 207499 is a potent inhibitor of antigen-induced bronchoconstriction in vivo. Moreover, SB 207499 suppresses both acute and persistent pulmonary eosinophilia under a variety of conditions. These antiallergic and anti-inflammatory activities are complemented by bronchodila-
tory activity at equivalent doses. All of these activities are produced without concomitant cardiovascular or overt CNS side effects. These characteristics make SB 207499 an outstanding candidate with which to evaluate clinically the anti-asthmatic activity of second-generation PDE4 inhibitors.

References


Beavo JA and Reifsnyder DH (1990) Primary sequence of cyclic nucleotide phospho-


Barnette MS, Christensen SB, Underwood DC and Torphy TJ (1996) Phosphodies-
terase 4Dm, a novel cAMP-specific phosphodiesterase inhibitor. J Pharmacol Exp Ther 278:1356–1361.

Christensen SB, Guider AM, Forster CJ, Gleason JG, Bender PB, Karpinski JM, Harris AL, Connell MJ, Ferguson EW, Wallace AM, Gordon RJ, Pagani ED and Howell RE, Sickles BD and Woeppel SL (1993) Pulmonary antiallergic and broncho-


Christensen SB, Guider AM, Forster CJ, Gleason JG, Bender PB, Karpinski JM, Harris AL, Connell MJ, Ferguson EW, Wallace AM, Gordon RJ, Pagani ED and Howell RE, Sickles BD and Woeppel SL (1993) Pulmonary antiallergic and broncho-

Giembycz MA and Raeburn D (1991) Putative substrates for cyclic nucleotide-


Dunn CJ, Elliot GA, Osztvene JA and Richards IM (1988) Development of a pro-


Giembycz MA and Raeburn D (1991) Putative substrates for cyclic nucleotide-


Harris AL, Connell MJ, Ferguson EW, Wallace AM, Gorden JD, Pagani ED and Silver FJ (1989) Role of low Km cyclic AMP phosphodiesterase in tracheal relax-


Howell RE, Sickles BD and Woeppel SL (1993) Pulmonary antiallergic and broncho-


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