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Identification of a Selective Nonpeptide Antagonist of the Anaphylatoxin C3a Receptor That Demonstrates Antiinflammatory Activity in Animal Models


The anaphylatoxin C3a is a potent chemotactic peptide and inflammatory mediator released during complement activation which binds to and activates a G-protein-coupled receptor. Molecular cloning of the C3aR has facilitated studies to identify nonpeptide antagonists of the C3aR. A chemical lead that selectively inhibited the C3aR in a high throughput screen was identified and chemically optimized. The resulting antagonist, N\(^2\)-[(2,2-diphenylethoxy)acetyl]-L-arginine (SB 290157), functioned as a competitive antagonist of \(^{125}\)I-C3a radioligand binding to rat basophilic leukemia (RBL)-2H3 cells expressing the human C3aR (RBL-C3aR), with an IC\(_{50}\) of 200 nM. SB 290157 was a functional antagonist, blocking C3a-induced C3aR internalization in a concentration-dependent manner and C3a-induced Ca\(^{2+}\) mobilization in RBL-C3aR cells and human neutrophils with IC\(_{50}\)s of 27.7 and 28 nM, respectively. SB 290157 was selective for the C3aR in that it did not antagonize the C5aR or six other chemotactic G protein-coupled receptors. Functional antagonism was not solely limited to the human C3aR; SB 290157 also inhibited C3a-induced Ca\(^{2+}\) mobilization of RBL-2H3 cells expressing the mouse and guinea pig C3aRs. It potently inhibited C3a-mediated ATP release from guinea pig platelets and inhibited C3a-induced potentiation of the contractile response to field stimulation of perfused rat caudal artery. Furthermore, in animal models, SB 290157, inhibited neutrophil recruitment in a guinea pig LPS-induced airway neutrophilia model and decreased paw edema in a rat adjuvant-induced arthritis model. This selective antagonist may be useful to define the physiological and pathophysiological roles of the C3aR.


The primary structure of human C3a was first determined in 1975 (10) and the site responsible for binding to its receptor, the carboxyl-terminal residues, was defined shortly thereafter (11). The C3aR was suspected to be a G protein-coupled receptor (GPCR) as C3a-induced increases in intracellular Ca\(^{2+}\) and C3-mediated release of reactive oxygen species in human polymorphonuclear leukocytes were blocked by pretreatment with pertussis toxin (12–14). This was confirmed when the C3aR was cloned in 1996 and definitively demonstrated to be a GPCR (15–17). The primary structure of the C3aR is unique among the superfamily of GPCRs. The receptor has been cloned from four different species, human, rat, mouse, and guinea pig, and all possess an unusually large second extracellular domain in excess of 170 aa long. Another striking and unanticipated feature of the C3aR is the tissue distribution pattern, where it is widely expressed throughout the periphery. There is also abundant expression of the C3aR transcript and protein in the brain (15, 18, 19).

Insight into the pathophysiological roles of C3a and the C3aR is emerging from gene targeting studies; two groups have independently produced C3aR\(^{-/-}\) mice by this approach (20, 21). C3aR\(^{-/-}\) mice are more susceptible than wild-type mice to an i.v.

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challenge with LPS, implicating complement activation and the C3aR in events leading to endothoxin-induced septic shock (21). Consistent with the effects of C3a on mast cells, eosinophils, and smooth muscle, allergen-challenged OVA-sensitized C3aR−/− mice do not exhibit airway hyperresponsiveness to methacholine challenge when compared with OVA-sensitized wild-type mice (20). Similarly, in a C3aR-deficient ibred strain of guinea pigs (C2BBR/R), which have a mutation encoding a stop codon within the coding sequence of the C3aR, we have noted significantly decreased bronchial reactivity in an OVA-induced asthma model (22). Allergen-challenged OVA-sensitized C2BBR/R guinea pigs exhibited a 30% decrease in bronchial reactivity when compared with allergen-challenged OVA-sensitized C2BBR/R guinea pigs which express an intact wild-type C3aR (22). These data obtained with Ag-sensitized C3aR−/− mice and guinea pigs implicate the C3aR in the pathogenesis of allergic asthma and suggest that C3aR antagonists may be useful in the treatment of this disease.

The lack of potent and selective C3aR antagonists has made it difficult to fully assess the relative contribution of C3a to the inflammatory processes elicited by the anaphylatoxins and the terminal complement complexes. Knowledge of the receptor-binding site of C3a and the molecular identification of the C3aR has facilitated discovery efforts to identify small molecule C3aR antagonists. We have used membranes from rat basophilic leukemia (RBL-2H3) cells stably expressing the human recombinant C3aR and radiolabeled C3a to establish a high throughput binding assay to screen for low m.w. nonpeptide C3aR antagonists. In this report, we describe the in vitro and preliminary in vivo pharmacological characterization of N3-[(2,2-diphenylethoxy)acetyl]-L-arginine (SB 290157), a potent and selective C3aR antagonist. Our data indicate that the compound should be a useful tool compound to help define the potential physiological role(s) of C3a.

Materials and Methods

Stable expression of C3aR in RBL-2H3 cells

RBL-2H3 cells (23), stably expressing the human, guinea pig, and mouse C3aRs, have previously been described (24, 25). The cell lines were maintained in Eagle’s MEM with Earle’s salts, with t-glutamine and nonessential amino acids (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS (HyClone, Logan, UT) and 400 μg/ml G418 (Life Technologies) in 10% DMSO working solution. The order of addition was 100 ng/ml laminin (Cambridge, MA) 96-well disposable chemotaxis plates (5 μm pore size). Varying concentrations of C3a with 3-ethyl-2-(3-pyridinylcarbonyl)glycl-L-arginine (SB 290936), a 4 μM fluo-3 acetoxymethyl ester (Molecular Probes, Eugene, OR) and 1.5 mM sulfipyrazone. Plates were incubated for 60 min at 37°C; medium was aspirated and replaced with the same medium without fluo-3 acetoxymethyl ester, and incubated for 10 min at 37°C. Cells were washed three times and incubated at 37°C for 100 μl assay buffer (120 mM NaCl, 4.6 mM KCl, 1.03 mM KH2PO4, 0.25 mM NaHCO3, 1.0 mM CaCl2, 1.1 mM MgCl2, 11 mM glucose, 20 μM HEPES (pH 7.4) with 1.5 mM sulfipyrazone). Plates were placed into FLIPR for analysis as described previously (29). The maximal change in fluorescence after agonist addition was quantitated. The percent of maximal C3a-induced Ca2+ mobilization was determined for each concentration of antagonist. The IC50, defined as the concentration of test compound that inhibits 50% of the maximal response induced by 1 nM C3a, was obtained from concentration-response curves. For agonist potency the EC20 was defined as the concentration that produces 50% of the maximal C3a-induced response.

Suspended cell Ca2+ mobilization

fura-2-acetoxymethyl ester-stored human neutrophils or RBL-C3aR cells were assayed for a Ca2+ mobilization response, as described (30).

Guinea pig platelet ATP release assay

A chemiluminescent assay was used to quantitate ATP release from C3a-stimulated guinea pig platelets, as described (31). Varying concentrations of antagonists were mixed with 1 nM C3a, a concentration equivalent to an EC40 in this assay, and the reaction was initiated by the addition of platelets.

HMC-1 chemotaxis assay

C3a-mediated chemotaxis of HMC-1 cells was assessed using Neuro Probe (Gaithersburg, MD) 96-well disposable chemotaxis plates (5 μm pore size). The top surface of the membrane was precoated with 100 ng laminin or fibronectin (Sigma, St. Louis, MO). Varying concentrations of C3a with and without antagonist were added in 28 μl RPMI 1640 to the lower wells. The filter was washed and assayed, and 2 to 5 × 105 cells were added in 25 μl to the top well. Plates were incubated at 37°C and 5% CO2 for 60 min. Filters were removed, and the top surfaces of the membranes were rinsed with PBS; then the cells were stained with Diff-Quik (Baxter, Dade Division, Gaithersburg, MD) and evaluated on a Beckman gamma counter 52500B (Beckman, Fullerton, CA). Data analysis was performed using KaleidaGraph v3.09 (Synergy Software, Reading, PA).
Miami, FL). The number of cells migrated was quantitated microscopically by counting the cells in three successive high power fields.

**Contraction of rat caudal artery**

Male Sprague Dawley normotensive rats weighing between 400 and 600 g were euthanized, and the tail was removed and placed in physiologic buffer. The tail was secured to a dissection board, the caudal artery was exposed, and a 30- to 40-mm-long section of the artery was dissected from the tail and placed into buffer. The artery section was cut into two segments of equal length; each segment was cannulated at both ends with PE50 tubing, and the tubing was secured with ties of 4-0 surgical silk. The cannulated arterial segments were mounted in a tubular glass chamber and were simultaneously perfused intraluminally and superfused extraluminally with oxygenated Krebs buffer at 38°C. The rate of intraluminal perfusion was 1 ml/min, and that of extraluminal superfusion was 2 ml/min. Under these conditions, the baseline perfusion pressure equilibrated to between 25 and 50 mm Hg. After a 20- to 30-min stabilization period, the periarterial sympathetic nerves were stimulated electrically every 30 s via platinum electrodes located at both ends of the chamber to obtain a brief, spike-like increase in perfusion pressure. The stimulation consisted of a 1-s train of square wave pulses at 70 V of 0.7 ms duration and a frequency of 15 Hz. These stimulation parameters resulted in a 50- to 100-mm Hg increase in perfusion pressure above baseline. When the response stabilized, one of the arterial segments was exposed to SB 290157 delivered in the superfusion flow, and the other artery was left untreated. After a 15-min exposure to SB 290157 (10 nM, 100 nM, and 1 μM), C3a (100 nM) was introduced in the superfusion flow to both arterial segments, and the effect on perfusion pressure was monitored. Typically, C3a enhanced the perfusion pressure. The C3a-mediated increase in perfusion pressure was rapidly desensitized (1–2 min).

**Guinea pig airway neutrophilia model**

Male Hartley guinea pigs were obtained from Charles River Breeding Laboratories (Raleigh, NC) and maintained in a barrier facility. Guinea pigs were placed four at a time into a plastic box (20 liters) that had been stirred for 30 min, the solution was warmed to ambient temperature. After stirring for 30 min, the reaction was quenched with water (20 ml), and the aequorein solution was extracted with ether (25 ml). The organic layer was washed with water (20 ml) and brine (20 ml). The organic solution was dried (MgSO4), and silica gel flash chromatography (3% ethyl acetate-hexanes) yielded (2S,2′R)-2,2′-diaryl-2′-methyl oxindole. The intermediate was treated with 25% trifluoroacetic acid (TFA)-CH2Cl2 for 1 h. The solven was removed, and the residual TFA was removed by azotrope with toluene to yield the title compound.

2-(2,2-Diphenylethoxy)acetic acid. Into a 0°C solution of 2,2-diphenylethan-1-ol (1 g) in DMF (10 ml) under argon was added aragon 60% sodium hydride (350 mg). The solution was stirred for 10 min, tert-butyl bromoacetate (884 μl) was added, and the solution was warmed to ambient temperature. After stirring for 30 min, the reaction was quenched with water (20 ml), and the aequorein solution was extracted with ether (25 ml). The organic layer was washed with water (20 ml) and brine (20 ml). The organic solution was dried (MgSO4), and silica gel flash chromatography (3% ethyl acetate-hexanes) yielded tert-butyl (2,2′-diphenylethoxy)acetate. The intermediate was treated with 25% trifluoroacetic acid (TFA)-CH2Cl2 for 1 h. The solvent was removed, and the residual TFA was removed by azotrope with toluene to yield the title compound.

**Pharmacokinetic studies in guinea pigs**

A pharmacokinetic study was conducted using three male Hartley guinea pigs. Under aseptic conditions, each guinea pig received surgically implanted femoral and arterial vein catheters at least 5 days before the study day. On the study day, fed animals received SB 290157 (30 mg/kg) as a single i.p. bolus injection (3 ml/kg total volume). The dose solution was prepared in normal saline (0.9% NaCl). Blood samples were obtained from a arterial catheter at various time intervals after administration of SB 290157; plasma was isolated by centrifugation. Plasma concentrations of SB 290157 were quantified by liquid chromatography/mass spectrometry (MS/MS) (lower limit of quantitation was 10 ng/ml). Noncompartamental methods were used for analysis of plasma concentration vs time data (35).

All animal experimental procedures were in accordance with protocols approved by the SmithKline Beecham Institutional Animal Care and Use Committee, and met or exceeded the standards of the American Association for the Accreditation of Laboratory Animal Care, the U.S. Department of Health and Human Services, and all local and federal animal welfare laws.

**Synthesis of SB 290157**

9-Fluorenylmethoxycarbonyl (F-moc)-arginine(Boc)-Wang. To F-moc-arginine(Boc) (1.8 g, 3 mmol) and Wang resin (2 g, 2 mmol) in CH2Cl2 (40 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (573 mg, 3 mmol) and 4-dimethylaminopyridine (244 mg, 2 mmol). The mixture was shaken overnight and washed twice with dimethylformamide (DMF) and six times with CH2Cl2.

2-(2,2-Diphenylethoxy)acetic acid. Into a 0°C solution of 2,2-diphenylethan-1-ol (1 g) in DMF (10 ml) under argon was added aragon 60% sodium hydride (350 mg). The solution was stirred for 10 min, tert-butyl bromoacetate (884 μl) was added, and the solution was warmed to ambient temperature. After stirring for 30 min, the reaction was quenched with water (20 ml), and the aequorein solution was extracted with ether (25 ml). The organic layer was washed with water (20 ml) and brine (20 ml). The organic solution was dried (MgSO4), and silica gel flash chromatography (3% ethyl acetate-hexanes) yielded tert-butyl (2,2′-diphenylethoxy)acetate. The intermediate was treated with 25% trifluoroacetic acid (TFA)-CH2Cl2 for 1 h. The solvent was removed, and the residual TFA was removed by azotrope with toluene to yield the title compound.

1H nuclear magnetic resonance (CDCl3) δ 7.1–7.4 (multiplet (m), 10H), 4.32 (triplet (t), J = 8.4 Hz, 1H), 4.0–4.1 (m, 4H).

N2-(2,2-Diphenylethoxy)acetic acid-1-arginine(Boc) (TFA-Wang). F-moc-2-arginine(Boc) (200 mg) was treated with 20% piperidine in CH2Cl2 (5 ml) for 30 min. The solvent was removed, and the resin was washed with CH2Cl2 (six times). To the resin in DMF (3.5 ml) was added (2,2-diphenylethoxy)acetic acid (92 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (69 mg), and 1-hydroxybenzotriazole (49 mg); and the mixture was shaken overnight and washed twice with dimethylformamide (DMF) and six times with CH2Cl2.

The resin was treated with a solution of 2.5% triisopropylsilane in 1:1 TFA-CH2Cl2 for 90 min. The cleavage residue was washed twice with hexanes, and the title compound was purified by reversed-phase HPLC (acetonitrile/water/0.1% trifluoroacetic acid). MS (electrospray) m/z = 413.3 (M+H)+.

**Results**

**In vitro activity**

To identify a nonpeptide C3aR antagonist, a high throughput radioligand binding assay was configured using membranes prepared from RBL-C3aR cells and [3H]-C3a. Approximately 240,000 compounds from the SmithKline Beecham compound collection were tested in a high throughput screen, affording 64 confirmed active compounds. One of these compounds, SKF 63649, was further progressed as a selective C3aR antagonist (Fig. 1A). Subsequent chemical optimization of this compound led to the discovery of SB 290157 (Fig. 1B). The affinity of the two compounds for the C3aR was evaluated in competitive binding experiments. SB 290157 was an order of magnitude higher affinity than SKF 63649 for the C3aR in this assay; the IC50 values were 200 and 3000 nM.
respectively (Fig. 2A). A related structure, SB 280936 (Fig. 1C), showed no affinity for this receptor in competitive binding assays at concentrations up to 10 μM and was used as a negative control.

To determine whether the compounds were functional antagonists, a FLIPR-based C3a-induced Ca\(^{2+}\) mobilization assay in RBL-C3aR cells was used. SKF 63649 and SB 290157 demonstrated concentration-dependent inhibition of 1 nM C3a-induced Ca\(^{2+}\) mobilization with IC\(_{50}\)s of 350 nM (n = 2) and 27.7 ± 2.9 nM, (n = 3), respectively (Fig. 2B). At concentrations up to 20 μM, SB 280936 had no effect on C3a-induced Ca\(^{2+}\) mobilization in RBL-C3aR cells. Testing activity with cells that naturally express the C3aR, we looked at the ability of the antagonists to inhibit C3a-induced Ca\(^{2+}\) mobilization in freshly isolated peripheral blood neutrophils. Both compounds were antagonists with IC\(_{50}\)s of 388 and 30 nM for SKF 63649 and SB 290157, respectively. SB 290157 was selective for the C3aR in that it did not antagonize C5a-induced Ca\(^{2+}\) mobilization in human neutrophils or in RBL-C5aR cells, nor did it inhibit Ca\(^{2+}\) mobilization responses for five other GPCRs on neutrophils, i.e., leukotriene B\(_4\), fMLP, platelet-activating factor, CXCR1, and CXCR2.

SB 290157 was evaluated for its ability to inhibit C3a-induced chemotaxis of HMC-1 cells, a human mast cell line that naturally expresses the C3aR and for which C3a is chemotactic (5, 6, 36). A concentration of 5 μM SB 290157 markedly inhibited C3a-mediated chemotaxis of HMC-1 cells (Fig. 2C). SB 290157 had no effect on C5a-mediated chemotaxis of HMC-1 cells (data not shown).

The antagonists were tested for inhibition of C3a-induced internalization of the C3aR. A 3-min incubation of neutrophils with 10 nM C3a is sufficient to stimulate internalization of ∼90% of the C3aR. Both SKF 63649 and SB 290157 inhibited C3aR internalization induced by 10 nM C3a in a concentration-dependent manner (Fig. 3). In the presence of >1 μM concentrations of the antagonists the internalization of the C3aR induced by C3a was reduced by ∼50% (Fig. 3). SB 280936 had no effect on C3aR internalization in this assay (Fig. 3).

In addition to functional antagonism of the human C3aR, SB 290157 also was a potent inhibitor of C3a-induced Ca\(^{2+}\) mobilization of RBL 2H3 cells stably expressing the mouse and guinea pig C3aRs (Table I). The IC\(_{50}\)s for SB 290157 inhibition of C3a-induced Ca\(^{2+}\) mobilization of the mouse and guinea pig C3aRs were 7 and 12.5 nM, respectively. SB 280936 was inactive at both the mouse and guinea pig C3aRs.

To assess the functional activity of the antagonists for endogenous C3aRs of species other than human, they were evaluated for the inhibition of 1 nM (EC\(_{80}\) concentration in this assay) C3a-induced ATP release from guinea pig platelets, cells that naturally express the C3aR (37). Both SKF 63649 and SB 290157 inhibited in a concentration-dependent manner with IC\(_{50}\) values of 385 ± 185 and 30 ± 14 nM, respectively (Table II).

At the tissue level, C3a is a spasmogen causing contraction of smooth muscle. SB 290157 was evaluated for the inhibition of C3a-induced contractile response to field stimulation of the perfused rat caudal artery. SB 290157 at concentrations of 100 nM and 1 μM completely abolished the effect of C3a, whereas a concentration of 10 nM was inactive (Fig. 4).

In vivo activity

The pharmacokinetic profile of SB 290157 was assessed in guinea pigs and mice after i.p. administration. The results of the guinea pig study are summarized in Fig. 5. When administered i.p. at a dose of 30 mg/kg, high and sustained plasma concentrations (>100 ng/ml, 0.25 μM) of SB 290157 were detected out to 8 h (Fig. 5). The C\(_{max}\) attained was 7000 ng/ml, and the apparent half-life (t\(_{1/2}\)) was 0.89 ± 0.26 h. Similar pharmacokinetic data were obtained after i.p. administration of SB 290157 to mice (t\(_{1/2}\) = 1.47 ± 0.10 h; data not shown).

FIGURE 1. Structures of SKF 63649 and analogs. A, SKF 63649; B, SB 290157; C, SB 280936 from this same chemical series but inactive and used as negative control.
SB 290157 was evaluated in a guinea pig LPS-induced airway neutrophilia model. As seen in Fig. 6, LPS (10 μg/ml) administered as an aerosol produced an infiltration of leukocytes (5-fold higher than with unexposed animals), especially neutrophils (~1000-fold) 48 h after LPS exposure. The resultant airway neutrophilia was reduced (39%) by administration of SB 290157, 30 mg/kg i.p. b.i.d. (LPS + vehicle = 33.2 ± 3.0 million neutrophils or 50.4% of total leukocytes recovered; LPS + SB 290157 = 20.3 ± 1.7 million neutrophils or 31.5% of total leukocytes; p = 0.02, Fisher’s protected least square difference). Total leukocyte numbers for treated animals were not significantly different (LPS + vehicle = 65.1 ± 11.3 million; LPS + SB 290157 = 62.0 ± 10.1 million) from vehicle-treated animals.

SB 290157 was also evaluated in an adjuvant-induced arthritis model using a prophylactic dosing protocol. Compound was administered to male Lewis rats starting on the day of adjuvant injection. SB 290157 was administered i.p. b.i.d., and paw inflammation was measured on day 20. There was 41% inhibition of paw edema on day 20 (p < 0.001) in animals that received a dose of 30 mg/kg b.i.d for 20 days. There was no significant effect on paw edema in rats that received 3 or 10 mg/kg SB 290157 b.i.d. (Fig. 7).

**Discussion**

A small molecule nonpeptide C3aR antagonist, SKF 63649, identified from a high throughput screen inhibited the C3aR binding with low micromolar affinity. After chemical optimization, to afford SB 290157, the affinity for the C3aR was increased by an order of magnitude. SB 290157 was a functional antagonist demonstrating equipotent inhibition of the C3a-induced Ca²⁺ mobilization response at the native receptor expressed on freshly isolated neutrophils, as well as at the recombinant C3aR stably expressed on RBL-2H3 cells. SB 290157 was a functional antagonist not only of the human C3aR but also of the mouse, rat, and guinea pig C3aRs. The potencies of SB 290157 for inhibition of C3a-induced Ca²⁺ mobilization of the mouse, guinea pig, and human receptors were similar (IC₅₀ = 7–30 nM). This was somewhat surprising in

<table>
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<th>RBL-2H3 Cells Expressing C3aR</th>
<th>SB 290157 IC₅₀ (nM)</th>
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<tbody>
<tr>
<td>Guinea pig</td>
<td>2.5 (n = 2)</td>
</tr>
<tr>
<td>Mouse</td>
<td>7 (n = 2)</td>
</tr>
<tr>
<td>Human</td>
<td>27.7 ± 2.9 (n = 3)</td>
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*The C3aR antagonist activity of SB 290157 was evaluated on the mouse and guinea pig C3aRs using the FLIPR-based Ca²⁺ assay in RBL-2H3 cells stably expressing these receptors. The IC₅₀, defined as the concentration of SB 290157 that inhibits 50% of the maximal response induced by 1 nM C3a, was obtained from concentration-response curves.*
light of the relatively low level of sequence identity (60–65% overall identity) between the C3aR from these different species (1). SB 290157 was selective for the C3aR and did not antagonize the C5aR or 5 other chemotactic GPCRs on human neutrophils.

There was good correlation between the antagonist potency of SB 290157 in the human neutrophil Ca\(^{2+}\) mobilization assay and the guinea pig ATP release assay. This result supports the recombinant receptor antagonist data demonstrating similar potency at endogenous C3aRs from two species. However, the antagonist potencies determined in the functional assays were ~7-fold higher than the affinity estimated in the whole cell binding assay. This is likely due to the inherent differences in the assay protocols, including: differences in times of incubation for the functional assays (seconds) vs the equilibrium conditions (30–60 min) in the binding assay; the temperatures at which the assays were run (room temperature for the binding assay vs 37°C for the functional assay); or possibly the effect of iodination on the affinity of C3a for its receptor. The effect of iodination of C3a on its interaction with the C3aR appears to be minimal, because the affinities determined for C3a with the C3aR in competition binding assays were in good agreement with the published $K_d$ for the C3aR (0.1–1.0 nM). In both binding and functional assays, SB 290157 was consistently 10-fold more potent as a C3aR antagonist than with the initial high throughput screening hit, SKF 63649.

The C3aR antagonist compounds had a significant effect on C3a-induced C3aR internalization, inhibiting by almost 50% the number of receptors internalized in response to challenge with 10 nM C3a. At doses of <10 μM, SB 290157 appeared to be a more potent antagonist of C3a-induced receptor internalization than SKF 63649, consistent with the potency obtained with this compound in the binding and functional assays.

Marked inhibition of C3a-mediated chemotaxis of HMC-1 cells and of the C3a-induced contractile response to field stimulation in perfused rat caudal arteries was also noted with SB 290157. Concentration response studies were difficult to perform in these assays, but SB 290157 antagonized mouse, rat, and guinea pig C3a receptors with potencies equivalent to the potency vs the human C3aR. These data, combined with the determination that after i.p. administration to mice and guinea pigs plasma levels of SB 290157 were high and sustained, indicated that it was a suitable compound for study in animal models to help define the physiological and pathophysiological role of C3a and the C3aR.

The pathophysiological role of C3a has been difficult to assess because as a result of complement activation both C3a and C5a are released into the circulation. Although C5a is generally more potent in its actions than C3a, the plasma concentration of C3a is much lower than the concentration of C5a. This makes C3a an attractive target for therapeutic intervention.

### Table II. Inhibition of C3a-mediated ATP release from guinea pig platelets

<table>
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<tr>
<th>Compound</th>
<th>IC(_{50}) (nM)</th>
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<tbody>
<tr>
<td>SB 280936</td>
<td>Inactive</td>
</tr>
<tr>
<td>SB 63649</td>
<td>385 ± 185</td>
</tr>
<tr>
<td>SB 290157</td>
<td>30 ± 14</td>
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</table>

* Varying concentrations of compounds were tested for the ability to inhibit the EC\(_{80}\) of C3a (~1 nM C3a) in the guinea pig platelet ATP release assay. The IC\(_{50}\) is the concentration that inhibited 50% of the 1 nM C3a response in absence of antagonist. Values of one typical experiment of three are presented.
reduction (41%) in paw swelling as compared with the control received SB 290157, 30 mg/kg i.p. b.i.d. There was a significant inhibition of the total number of cells recovered. This is somewhat surprising because C3a is not chemotactic for neutrophils, whereas C3a administered by coronary bolus to guinea pigs in vivo resulted in neutropenia in Lewis rats. Rats were treated with SB 290157 in 5% ethanol, 10% Cremaphor-El, and 85% saline at 3, 10, and 30 mg/kg i.p. b.i.d. or vehicle (AIA cont) alone starting on the day of adjuvant injection, and paw edema was measured on day 20 as described in Materials and Methods. Values are the mean ± SEM of 10–12 rats/group (inhibition expressed as % AIA controls). * p < 0.001 vs AIA controls.

FIGURE 7. Prophylactic activity of SB 290157 on paw edema in AIA in Lewis rats. Rats were treated with SB 290157 in 5% ethanol, 10% Cremaphor-El, and 85% saline at 3, 10, and 30 mg/kg i.p. b.i.d. or vehicle (AIA cont) alone starting on the day of adjuvant injection, and paw edema was measured on day 20 as described in Materials and Methods. Values are the mean ± SEM of 10–12 rats/group (inhibition expressed as % AIA controls). * p < 0.001 vs AIA controls.

significantly higher (~10-fold) than that of C5a (38). Another factor complicating studies of the in vivo actions of C3a is the rapid inactivation of this peptide via the cleavage of the amino-terminal arginine residue by carboxypeptidase N. The resultant peptide, C3a(desArg), is inactive at the C3aR (13, 39), therefore serum carboxypeptidase N inhibitors have been used for in vivo studies to increase the effectiveness of C3a (40). Several groups have assessed the pulmonary and cardiac effects of C3a in animals after direct administration. Intrabronchial instillation of C3a into guinea pigs induced acute pulmonary injury and bronchospasm (8, 9). The i.v. injection of C3a into guinea pigs caused a rapid neutropenia with resultant sequestration of neutrophils within lung tissue (7), whereas C3a administered by coronary bolus to guinea pigs induced cardiac dysfunction (41).

Recent reports with C3aR-deficient guinea pigs showed decreased bronchial reactivity in a OVA-induced asthma model compared with control animals (22). In addition, another recent study with C3aRa−/− mice has demonstrated decreased airway hyperresponsiveness to methacholine challenge compared with wild-type mice (20). These studies suggested a potential role for C3a in inflammatory pulmonary diseases such as asthma and acute respiratory distress syndrome.

We studied the C3aR antagonist, SB 290157, in two animal models of inflammation. In the first, SB 290157 inhibited neutrophil recruitment and accumulation in a guinea pig LPS-induced airway neutrophilia model. The inhibitory activity appeared to be specific for neutrophils as the number of neutrophils recovered in the challenged lungs was decreased, but there was no significant inhibition of the total number of cells recovered. This is somewhat surprising because C3a is not chemotactic for neutrophils, although they express the C3aR, demonstrate specific binding, and respond to C3a with a transient calcium response (27). The effect of SB 290157 may be a secondary rather than a direct effect on neutrophil recruitment.

SB 290157 was also tested in a disease-modifying rat model of AIA. Antiinflammatory activity was observed in Lewis rats that received SB 290157, 30 mg/kg i.p. b.i.d. There was a significant reduction (41%) in paw swelling as compared with the control untreated animals. This is significant activity for the C3aR antagonist in an aggressive arthritis model and potentially implicates C3a in the pathogenesis of this disease.

Our data indicate that SB 290157 is a high affinity, selective, and competitive C3aR antagonist. It is active in two in vivo models of inflammation; therefore, it shows promise as a tool compound for further studies to elucidate physiological and pathophysiological role(s) of C3aR activation.

References


