

RESEARCH PAPER

In vitro and *in vivo* characterization of A-940894: a potent histamine H₄ receptor antagonist with anti-inflammatory properties

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Background and purpose: The histamine H₄ receptor is widely expressed in cells of immune origin and has been shown to play a role in a variety of inflammatory processes mediated by histamine. In this report, we describe the *in vitro* and *in vivo* anti-inflammatory properties of a potent histamine H₄ receptor antagonist, A-940894 (4-piperazin-1-yl-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-d]pyrimidin-2-ylamine).

Experimental approach: We have analysed the pharmacological profile of A-940894 at mouse native, rat recombinant and human recombinant and native, histamine H₄ receptors by radioligand binding, calcium mobilization, mast cell shape change, eosinophil chemotaxis assays and in the mouse model of zymosan-induced peritonitis.

Key results: A-940894 potently binds to both human and rat histamine H₄ receptors and exhibits considerably lower affinity for the human histamine H₁, H₂ or H₃ receptors. It potently blocked histamine-evoked calcium mobilization in the fluorometric imaging plate reader assays and inhibited histamine-induced shape change of mouse bone marrow-derived mast cells and chemotaxis of human eosinophils *in vitro*. In a mouse mast cell-dependent model of zymosan-induced peritonitis, A-940894 significantly blocked neutrophil influx and reduced intraperitoneal prostaglandin D₂ levels. Finally, A-940894 has good pharmacokinetic properties, including half-life and oral bioavailability in rats and mice.

Conclusions and Implications: These data suggest that A-940894 is a potent and selective histamine H₄ receptor antagonist with pharmacokinetic properties suitable for long-term *in vivo* testing and could serve as a useful tool for the further characterization of histamine H₄ receptor pharmacology.

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Keywords: histamine; H₄; selective antagonist; A-940894; receptor binding; mast cells; eosinophils; shape change; chemotaxis; zymosan

Abbreviations: A-940894, (4-piperazin-1-yl-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-d]pyrimidin-2-ylamine); ABT-239, [4-(2-{2-[(2R)-2-methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile; JNJ777120, 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine; C_{max}, maximum concentration; FLIPR, fluorometric imaging plate reader; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HEK293, human embryonic kidney cells; MPO, myeloperoxidase; PGD₂, prostaglandin D₂; qRT-PCR, quantitative RT-PCR; V_d, volume of distribution

Introduction

The histamine H₄ receptor is the most recently identified member of the G-protein coupled histamine receptor family,

displaying distinct molecular and pharmacological properties from H₁, H₂ and H₃ histamine receptor subtypes (Oda *et al.*, 2000; Morse *et al.*, 2001; nomenclature follows Alexander, 2008). The mRNA for H₄ receptors is expressed in a number of peripheral tissues, including bone marrow and spleen (Oda *et al.*, 2000; Morse *et al.*, 2001) and at the cellular level, histamine H₄ receptor mRNA is also expressed by several immune cells, including eosinophils, monocytes, neutrophils, mast cells, dendritic cells, basophils and T-cells (Oda *et al.*, 2000; Morse *et al.*, 2001; Damaj *et al.*, 2007) where the

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histamine H₄ receptor may play a role in chemotactic and inflammatory processes (Thurmond *et al.*, 2008), although the precise function of histamine H₄ receptors in regulating inflammation is not well understood.

Histamine H₄ receptors have been implicated in rodent models of acute inflammation, including zymosan-induced peritonitis and experimental colitis as well as in acute inflammatory pain (G.C. Hsieh *et al.*, unpubl. experiments; Takeshita *et al.*, 2004; Thurmond *et al.*, 2004; Varga *et al.*, 2005). In a rodent asthma model, blockade of H₄ receptors reduced eosinophil infiltration while mast cell redistribution and eosinophilia have been associated with allergic rhinitis (Fokkens *et al.*, 1992; Slater *et al.*, 1996). Thus, it has been postulated that diseases associated with immune cell-mediated responses, such as asthma, allergic rhinitis, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases, may be potential targets for treatment with H₄ receptor antagonists (Daugherty, 2004; Fung-Leung *et al.*, 2004; Zhang *et al.*, 2006; Thurmond *et al.*, 2008).

Thioperamide, originally characterized as a histamine H₃ receptor antagonist, was the first H₄ antagonist identified when it was shown to block H₄ receptor-mediated functional properties (Liu *et al.*, 2001a; Morse *et al.*, 2001), although at concentrations that also antagonize H₃ receptors. The discovery of JNJ777120 (1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine) as the first orally active, potent and selective non-imidazole H₄ receptor antagonist (Jablonowski *et al.*, 2003) allowed a significant advance in the understanding of the pharmacology and function of H₄ receptors both in *in vitro* and *in vivo* (Thurmond *et al.*, 2004). Other selective, non-imidazole H₄ antagonists, such as the benzimidazole analogue VUF6002, have also been recently described (Coruzzi *et al.*, 2007). More recently, structurally different non-imidazoles, such as A-943931 and A-987306 (Coward *et al.*, 2008; Liu *et al.*, 2008; D. G. Witte *et al.*, unpubl. experiments), have been shown to behave as potent and selective H₄ receptor antagonists *in vitro* and *in vivo*. The aim of this report is to describe the pharmacological properties of A-940894, a potent and selective H₄ receptor antagonist with favourable pharmacokinetic and *in vivo* anti-inflammatory properties in rodents.

Methods

Animals

Animals for experiments conducted in house were housed in Association for Assessment and Accreditation of Laboratory Animal Care-approved facilities at Abbott Laboratories in a temperature-regulated environment with lights on between 6:00 AM and 6:00 PM. All experimental protocols were approved by Abbott's Institutional Animal Care and Use Committee. Male Sprague-Dawley rats were supplied by Charles River Laboratories (Portage, MI, USA). Female BALB/c mice were obtained from Taconic (Hudson, NY, USA), while WBB6F1/J-Kit^W/Kit^{W-v} (W/W^v mast cell deficient mice) and their normal controls, WBB6F1/J-Kit^W, were from Jackson Laboratories (Bar Harbor, ME, USA). The animals were acclimated to laboratory conditions for at least 1 week before testing.

Cloning and expression of histamine H₄ receptors

All reagents used for cell culture were acquired from Invitrogen (Carlsbad, CA, USA) unless otherwise indicated. Human embryonic kidney (HEK293) cells were from ATCC (Manassas, VA, USA). Full-length human histamine H₄ receptor cDNA was cloned using total RNA isolated from the HL-60 human promyelocytic leukaemia cell line (ATCC, Manassas, VA, USA) with RT-PCR methods using primers designed according to the published human H₄ receptor gene sequences to give a sequence identical to accession # AY136745. Full-length rat and mouse H₄ receptor cDNAs were cloned using either rat spleen poly-A⁺ (Clontech, Mountain View, CA, USA) or mouse bone marrow total RNA (Zyagen, San Diego, CA, USA) to give sequences identical to accession # AF358860 and AF358859 respectively. All cDNAs were subcloned into the pCI-neo expression vector (Promega, Madison, WI, USA). Human, rat and mouse vectors were transfected into a clonal host HEK293 cell line stably expressing the chimeric G α_{q15} protein, to re-direct histamine H₄ receptor signalling to intracellular calcium release (Yao *et al.*, 2003), using LIPO-FECTAMINE 2000 (Invitrogen, Carlsbad, CA, USA). Stable cell lines expressing these receptors were established using standard molecular techniques and were used in a fluorometric imaging plate reader (FLIPR) assay. In addition to that, rat histamine H₄ receptor cDNA was subcloned into pFLAG-CMV-4 (Sigma, Saint Louis, MO, USA) and stable cell line expressing a FLAG-tagged rat H₄ receptors (rat H₄-FLAG) was also established using HEK293 cells. These cell lines were used in radioligand and [³⁵S]-GTP γ S binding assays.

Membrane preparations

Cells expressing histamine H₄ receptors were harvested and homogenized in TE buffer (50 mmol·L⁻¹ Tris-HCl, 5 mmol·L⁻¹ EDTA, pH 7.4) using a Polytron at 20 000 r.p.m. for 2 × 10 s bursts in the presence of protease inhibitors [1 mmol·L⁻¹ benzamidine, 2 µg·mL⁻¹ aprotinin, 1 µg·mL⁻¹ leupeptin and 1 µg·mL⁻¹ pepstatin (Sigma-Aldrich)], followed by centrifugation at 40 000× *g*. The homogenization and centrifugation steps were repeated as described above to further purify the membrane pellets. Final membrane preparations were obtained by re-homogenizing the pellets in 6.25 volumes (weight/volume) of TE buffer and frozen at -70°C until used. For the rat H₄-FLAG receptor cell lines, final membrane preparations included glycerol and bovine serum albumin (BSA) at a final concentration of 10% and 1% respectively.

Radioligand binding assays

For competition binding, membrane preparations from cells expressing human histamine H₄ receptors or rat H₄-FLAG receptors were incubated with [³H]-histamine (12–20 nmol·L⁻¹) in the presence or absence of increasing concentrations of ligands for H₄ receptors. Incubations were in a final volume of 0.5 mL TE buffer at 25°C and were terminated after 30 min by rapid filtration. Non-specific binding was determined in presence of 10 µmol·L⁻¹ JNJ777120. In saturation binding experiments *B*_{max} of the human H₄ receptor cell line was found to be 216 fmol·mg⁻¹ and *K*_d of [³H]-histamine 26 nmol·L⁻¹ (*pK*_d = 6.83 ± 0.09). For the rat H₄-FLAG cell line

$B_{\max} = 1624 \text{ fmol} \cdot \text{mg}^{-1}$ and $K_d = 109 \text{ nmol} \cdot \text{L}^{-1}$ ($\text{p}K_d = 7.70 \pm 0.04$, data not shown). Radioligand binding assays for cloned human histamine H₁, H₂ and H₃ receptors were performed as described (Strakhova and Esbenshade, 2006) using [³H]-mepyramine, [³H]-tiotidine and [³H]-N- α -methylhistamine respectively. All binding reactions were terminated by filtration under vacuum onto polyethylenimine (0.3%) pre-soaked Unifilters (Perkin Elmer Life Sciences, Boston, MA, USA) or Whatman GF/B filters (for histamine H₄ receptors) followed by three brief washes with 2 mL of ice-cold TE buffer. Bound radiolabel was determined by liquid scintillation counting.

For all of the radioligand competition binding assays, IC₅₀ values and Hill slopes were determined by Hill transformation of the data as previously described (Strakhova and Esbenshade, 2006) and $\text{p}K_i$ values were determined by the Cheng–Prusoff equation (Cheng and Prusoff, 1973) using K_d values derived from saturation studies.

[³⁵S]-GTP γ S binding assays

For assays to determine antagonist activity, membranes expressing rat H₄-FLAG receptors were diluted in GTP γ S assay buffer (25 mmol·L⁻¹ HEPES, 2.5 mmol·L⁻¹ MgCl₂ and 75 mmol·L⁻¹ NaCl, pH 7.4) and 10 μ g of membrane protein was incubated in a 96-well deep-well block in the presence of 5.0 mmol·L⁻¹ unlabelled GDP, approximately 0.5 nmol·L⁻¹ of [³⁵S]-GTP γ S, histamine (10 μ mol·L⁻¹) and increasing concentrations of H₄ receptor antagonists. Samples were incubated at 37°C for 5 min. The assays were terminated by the addition of cold buffer (50 mmol·L⁻¹ Tris-HCl, 75 mmol·L⁻¹ NaCl and 2.5 mmol·L⁻¹ MgCl₂, pH 7.6) and subsequent harvesting onto a Packard Unifilter 96-well GF/B plate from Perkin Elmer Life Sciences. After extensive washing, the plates were dried, Microscint 20 was added to the samples and the amount of bound [³⁵S]-GTP γ S was determined utilizing the Topcount (Perkin Elmer Life Sciences). The percentage of [³⁵S]-GTP γ S bound in each sample was calculated as a percentage of that bound to control samples (basal or histamine-stimulated) incubated in the absence of histamine H₄ receptor ligands. Triplicate determinations were obtained at each concentration and the data were analysed using GraphPad Prism (San Diego, CA, USA) to obtain IC₅₀ values and Hill slopes. K_b values were calculated from IC₅₀ values using the generalized Cheng–Prusoff equation (Cheng and Prusoff, 1973; Leff and Dougall, 1993). The mean with 95% confidence limits of at least three independent experiments is reported.

Measurement of intracellular calcium levels

Intracellular calcium responses were measured in cell lines co-expressing G α_{q15} and either human, rat or mouse H₄ receptors, using FLIPR methods as previously described, with modifications (Esbenshade *et al.*, 2003). Briefly, the FLIPR assay was modified by setting the FLIPR temperature to 37°C, with cell plates and drug plates pre-warmed in a 37°C incubator for 10 min before placement in the FLIPR. Chlorpheniramine (10 μ mol·L⁻¹) was added to the incubation buffer to mask any H₁ receptor-mediated responses. Antagonist potencies (K_b) were obtained by measuring inhibition of a reference response (1000, 300 and 100 nmol·L⁻¹ histamine for rat,

mouse and human H₄ cell lines respectively). Antagonists were added 5 min prior to addition of histamine. Peak response values for the antagonist wells were expressed as a percentage of the reference peak response for histamine in the absence of H₄ receptor antagonists. Experiments were performed in duplicate wells, and data were analysed using GraphPad Prism to obtain IC₅₀ values. The generalized Cheng–Prusoff equation (Cheng and Prusoff, 1973; Leff and Dougall, 1993) was used to determine K_b values, which are presented as the mean with 95% confidence limits.

Eosinophil chemotaxis assay

Venous blood from healthy volunteers was sampled according to local ethics committee-approved protocol. To determine the ability of compounds to block histamine H₄ receptor-mediated chemotaxis of human eosinophils, eosinophils from individual subjects were isolated and processed separately following the isolation as outlined below. Aliquots of heparin-treated whole venous blood were slowly layered over 20 mL of Polymorphprep (Accurate Chemical and Scientific Corp., Westbury, NY, USA) and centrifuged at 870 \times *g* for 25 min at 4°C. The upper platelet rich fraction and the upper mononuclear cell band were discarded, while the polymorphonuclear cell band and the lower 'red zone' were collected. The collected cells were washed in cold autoMACS[®] rinsing solution (Miltenyi Biotec, Auburn, CA, USA) containing 0.5% BSA and centrifuged at 1150 \times *g* for 5 min at 4°C. Following centrifugation, supernatants were decanted and the red cells were lysed in 500 μ L H₂O for 30 s. Immediately following lysis, cells were diluted in cold rinsing solution and lysis was repeated as above until all red cells were depleted. After the final lysis, cells were resuspended in cold rinsing solution with 0.5% BSA at a final concentration of 1×10^6 cells· μ L⁻¹ and labelled for at least 30 min with an equal volume of cold CD16 magnetic microbeads (Miltenyi Biotec) at 4°C. After labelling, cells were washed and resuspended in 500 μ L cold rinsing solution with 0.5% BSA. Eosinophils were purified through MACS[®] CS separation columns (Miltenyi Biotec) by negative selection. Eosinophils (which do not bind to the magnetic CD16 microbeads) were eluted with 30 mL of cold RPMI 1640 media (Invitrogen) with 0.5% BSA and centrifuged at 300 \times *g* for 10 min at 4°C. Cell pellet was resuspended at 1.2×10^6 – 2×10^6 cell·mL⁻¹ in warm RPMI 1640 with 0.5% BSA. In order to verify purity, cell aliquots were stained using an eosinophil stain kit (ENG Scientific, Inc. Clifton, NJ, USA).

To measure eosinophil chemotaxis, the bottom chamber of the chemotaxis plate was filled, in quintuplicate, with 300 μ L of RPMI 1640 with 0.5% BSA (vehicle), 1 μ mol·L⁻¹ histamine alone or test compounds in 1 μ mol·L⁻¹ histamine, and was separated from the top chamber by a 5 μ m filter (Neuro Probe, Gaithersburg, MD, USA) coated with 100 ng·mL⁻¹ fibronectin. Purified eosinophils were resuspended at 60 000–100 000 cells in 50 μ L of RPMI 1640 with 0.5% BSA with or without test compounds and added to the top of the chamber. Chemotaxis plates were incubated for 90 min in a 37°C 5% CO₂ incubator. Eosinophil migration from the top chamber to the bottom chamber was quantified using Cell Titer-Glo[®] Luminescent Cell Viability Assay (Promega, Madison, WI, USA). Data were analysed using Prism GraphPad software.

Zymosan-induced peritonitis

Female BALB/c mice were used to establish zymosan-induced peritonitis and study the effects of histamine H₄ receptor antagonists. JNJ777120 and A-940894 were given at 1–100 mg·kg⁻¹ s.c. (*n* = 5 per group) in saline 30 min prior to i.p. administration of 2 mg zymosan (Sigma-Aldrich) in saline (0.5 mL per mouse). For analysis of histamine and cytokine content, animals were killed 1.5 h after the challenge, and the lavage fluids were collected by washing peritoneal cavities with 3 mL of phosphate-buffered saline (pH 7.2) with 10 units heparin·mL⁻¹. The samples were centrifuged at ~100× *g*, 4°C for 5 min and supernatants were removed and frozen for future analysis. Dexamethasone at a dose of 10 mg·kg⁻¹ (dissolved in water) was also included in the same study. For analysis of neutrophil influx and RNA, animals were killed at various time points after the zymosan challenge, lavages were conducted as described above and cell pellets were preserved. Dexamethasone-induced inhibition of myeloperoxidase (MPO) and prostaglandin (PG)D₂ levels was considered a full response.

Assay of MPO activity

The lavage samples were centrifuged at ~100× *g*, 4°C for 5 min. The cell pellets were brought up in 1 mL of 50 mmol·L⁻¹ potassium phosphate buffer (pH 6.0) containing 0.5% (hexadecyltrimethylammonium bromide), freeze-thawed three times, and centrifuged at ~100× *g* for 10 min at room temperature. At this point the pellets were discarded and supernatants were frozen and stored at -70°C until future use. MPO assays were performed as described previously (Bradley *et al.*, 1982) with some modifications to accommodate a 96-well format. The reaction was initiated by the addition of assay buffer containing tetramethylbenzidine (Sigma-Aldrich, St. Louis, MO), allowed to proceed for 15 min at room temperature, then stopped with 1 mol·L⁻¹ H₂SO₄. The absorbance was read at 450 nm·L⁻¹.

Determination of histamine and prostaglandin D₂ content

Concentration of histamine in the peritoneal lavages was determined using histamine EIA kit (IBL, Minneapolis, MN, USA) according to the manufacturer's recommendations. Prostaglandin D₂ (PGD₂) was assayed using EIA kits from Cayman Chemicals (Ann Arbor, MI, USA) following the manufacturer's recommendations.

RNA isolation and real-time quantitative RT-PCR

For analysis of H₄ mRNA expression, lavages were conducted as described above and were flash-frozen and stored at -70°C. Samples were crushed and homogenized in 5 mL Trizol and 5 µL RNaseout (Invitrogen). RNA was isolated using Trizol according to the manufacturer's protocol. Quantitative determination of RNA expression levels was performed by quantitative RT-PCR (qRT-PCR) TaqMan® technology. Gene Expression Assays (Applied Biosystems, Foster City, CA, USA), containing primers and FAM™ dye-labelled TaqMan® MGB probe were used to detect mouse histamine H₄ receptor (Mm00467633_ml) and mouse glyceraldehyde-3-phosphate

dehydrogenase (GAPDH) (Mm99999915_g1). For qRT-PCR analysis, RNA was converted into cDNA using the ABI high-capacity cDNA reverse transcription kit. cDNA was run in a 20 µL reaction using Taqman Universal PCR Master Mix (Invitrogen). Cycling parameters: 10 min at 95°C, and then 45 cycles of 15 s at 95°C, 1 min at 60°C. Data were collected during each extension phase of the PCR reaction and analysed with the ABI-7000 SDS software package (Applied Biosystems). Each sample was run in triplicate and normalized using the GAPDH data.

Bone marrow mast cell culture

The femurs from BALB/c female mice (aged 8–10 weeks) were flushed with cold RPMI 1640. Cells were collected, filtered through a 100 µm filter and centrifuged (~250× *g*, 5 min). After treatment with PharmLyse (Pharmingen, San Jose, CA, USA) to lyse red blood cells, the cells were washed twice with cold RPMI 1640 and plated at 1 × 10⁵–10⁶ cells·mL⁻¹ in complete RPMI medium (RPMI 1640 containing 5 mmol·L⁻¹ glutamine, 0.1 mmol·L⁻¹ non-essential amino acids, 160 units·mL⁻¹ penicillin, 160 µg·mL⁻¹ streptomycin, 50 µmol·L⁻¹ β-mercaptoethanol and 10 ng·mL⁻¹ recombinant murine IL-3). After each week, non-adherent cells were removed, centrifuged (~250× *g*, 5 min), fresh media added and transferred to a new dish for further culture. After 4 weeks, cells were analysed by flow cytometry for FcεRI and c-kit (CD117) expression. Mast cells from 4–7 week cultures were >99% positive for both FcεRI and c-kit and were used in gated autofluorescence forward scatter experiments.

Shape change (gated autofluorescence forward scatter) assay

Cells prepared as described above were centrifuged (~250× *g*, 5 min) and resuspended at 2 × 10⁶ cells·mL⁻¹ in RPMI 1640 + 0.1% BSA. A total of 140 µL of cells were incubated in the presence or absence of antagonists for 5 min at 37°C. Cells were stimulated with histamine (10 µmol·L⁻¹ final concentration) for 10 min at 37°C, then fixed with 1.5% paraformaldehyde on ice to maintain cell shape. Fluorescence was measured by flow cytometry (Becton Dickinson FACSCaliber, Becton Dickinson, San Jose, CA, USA). Bone marrow mast cells were gated by their autofluorescence and 10000 events were acquired for each sample. Results were calculated as % increase in forward scatter compared with unstimulated cells. Raw data were analysed using GraphPad Prism® 4 software. IC₅₀ values for antagonists were calculated using non-linear regression and K_b values calculated using a modified Cheng-Prusoff equation (Leff and Dougall, 1993).

Pharmacokinetics

Male Sprague-Dawley-derived rats, weighing 250–400 g, were obtained from Charles Rivers Laboratories. The animals were fasted overnight prior to dosing and throughout the first 8 h of the study period. Dosing solutions were prepared as a 10 mg·mL⁻¹ solution in 10% EtOH: 5% dextrose in water. All animals received a 10 mg·kg⁻¹ (1 mL·kg⁻¹) dose by i.v. or oral administration. Samples were analysed in our Exploratory Kinetics and Analysis Department. Briefly, compounds were

selectively removed from the plasma using protein precipitation with acetonitrile at neutral pH. The samples were vortexed vigorously followed by centrifugation. The supernatant was evaporated to dryness with a gentle stream of nitrogen over low heat (~35°C). The samples were reconstituted by vortexing with mobile phase. A-940894 and its internal standard were separated from co-extracted contaminants on a 50 mm × 3 mm 5 µm Luna CN column with an acetonitrile/1% acetic acid/0.1% trifluoroacetic acid (47.6:46.0:6.4, by volume) mobile phase at a flow rate of 0.4 mL·min⁻¹ with a 25 µL injection. Compounds were quantified using MRM detection with a turbo ionspray source on a mass spectrometer (PE Sciex Applied Biosystems, Foster City, CA). The limit of quantification was approximately 13 ng·mL⁻¹ for rat plasma samples. Plasma binding was determined using rat whole plasma.

Materials

A-940894 (4-piperazin-1-yl-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-d]pyrimidin-2-ylamine) was synthesized at Abbott Laboratories as previously described (Altenbach *et al.*, 2008). ABT-239 ([4-(2-[2-[(2R)-2-methylpyrrolidinyl]ethyl]-benzofuran-5-yl)benzonitrile) was synthesized at Abbott Laboratories (Abbott Park, IL, USA). JNJ7777120 was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). [³H]-histamine (30–60 Ci·mmol⁻¹) was from Amersham Biosciences Inc. (Piscataway, NJ, USA). [³H]-N-(methylhistamine (45–90 Ci·mmol⁻¹), [³H]-pyrilamine (20–30 Ci·mmol⁻¹), [³H]-tiotidine (70–90 Ci·mmol⁻¹), [³H]-histidine (40–60 Ci·mmol⁻¹) and [³⁵S]-GTP(S (1250 Ci·mmol⁻¹) were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA, USA). Thioperamide was purchased from Tocris Cookson Inc. (Ellisville, MO, USA); histamine, loratadine, terfenadine and cimetidine were from Sigma-Aldrich.

Results

Radioligand binding competition studies

The histamine H₄ receptor antagonist A-940894 (Figure 1) exhibited high affinity for recombinant human and rat H₄ receptors with respective K_i values of 71 and 7.6 nmol·L⁻¹ (Table 1). This compound is highly selective over the other histamine receptor subtypes; for example, it exhibits nearly 70-fold selectivity for the human H₄ receptor versus human

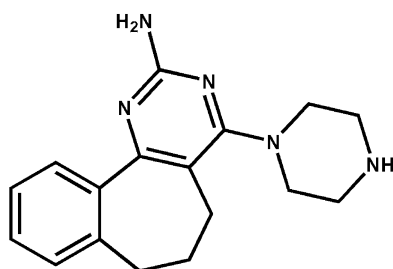


Figure 1 Chemical structure of A-940894 (4-piperazin-1-yl-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-d]pyrimidin-2-ylamine).

H₁ and H₂ receptors (K_i > 5000 nmol·L⁻¹) and also exhibits lower affinity for human (K_i = 3900 nmol·L⁻¹) and rat (K_i = 483 nmol·L⁻¹) H₃ receptors. Additional selectivity of A-940894 was evaluated across a panel of over 85 receptors, kinases and ion channels (CEREP and in-house). At 10 µmol·L⁻¹, A-940894 inhibited specific binding at the following human receptors: β₁-adrenoceptors (77%), M₄ muscarinic (83%), µ-opioid (90%), several 5-HT receptors, namely 5-HT_{1A} (92%, K_i = 890 nmol·L⁻¹), 5-HT_{1B} (99%), 5-HT₃ (98%) receptors and the 5-HT transporter (93%). JNJ7777120 displayed high affinity towards recombinant human and rat histamine H₄ receptors with K_i values of 12 and 3.1 nmol·L⁻¹ respectively, but low affinity towards H₁, H₂ and H₃ receptors (K_i > 1000 nmol·L⁻¹).

Recombinant H₄ receptor functional antagonism

The antagonist profile of A-940894 was assessed by measuring intracellular calcium mobilization in cell lines co-expressing human, rat and mouse H₄ receptors along with the chimeric G-protein, Gα_{q15}. Histamine gave robust dose-dependent responses with EC₅₀ values of 17 nmol·L⁻¹ (pEC₅₀ = 7.77 ± 0.01) for human, 328 nmol·L⁻¹ (pEC₅₀ = 6.49 ± 0.01) for rat and 50 nmol·L⁻¹ (pEC₅₀ = 7.23 ± 0.04) for mouse H₄ receptor cell lines respectively. A-940894 blocked histamine-evoked intracellular calcium responses at human, rat and mouse H₄ receptor cell lines with K_b values of 74, 15 and 18 nmol·L⁻¹ respectively (Table 2) while JNJ7777120 provided K_b values ranging from 2.7 to 4.7 nmol·L⁻¹ at the three species. The functional character of A-940894 was also determined in a [³⁵S]-GTPγS binding assay using membranes from cells expressing the rat H₄-FLAG receptor. Histamine stimulated basal [³⁵S]-GTPγS binding in a dose-dependent manner, with a mean EC₅₀ value of 2.6 µmol·L⁻¹, producing maximal efficacy of 208% of basal. A-940894 had no effect on basal [³⁵S]-GTPγS binding up to a concentration of 100 µmol·L⁻¹ and as such, did not display agonist or inverse agonist activity in this assay, but did display dose-dependent inhibition (K_b = 60 nmol·L⁻¹) of histamine-stimulated [³⁵S]-GTPγS binding (Table 2). JNJ7777120 exhibited dose-dependent inhibition of histamine-stimulated [³⁵S]-GTPγS binding (K_b = 62 nmol·L⁻¹).

Table 1 Binding affinities of A-940894 and JNJ7777120 at recombinant human or rat histamine H₁, H₂, H₃ and H₄ receptors

Receptor subtype	K _i (nmol·L ⁻¹)	
	A-940894	JNJ7777120
Human H ₄	71 ^a (26–196) ^b	12 (2–63)
Rat H ₄	7.6 (5.1–11.4)	3.1 (1.6–6.0)
Human H ₁	>5000	2767 (1087–7048)
Human H ₂	>5000	>5000
Human H ₃	3899 (2582–5902)	2210 (1696–2882)
Rat H ₃	483 (333–702)	1382 (1083–1764)

^an ≥ 3.

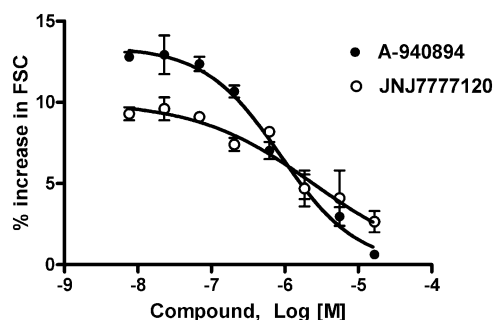
^b95% confidence interval.

Table 2 Functional properties of A-940894 and JNJ777120

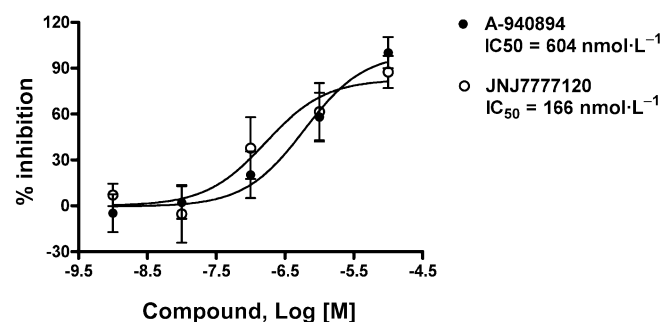
Receptor and assay	K _b (nmol·L ⁻¹)	
	A-940894	JNJ777120
Human H ₄ – FLIPR	74 ^a (60–92) ^b	4.7 (4.4–5.0)
Rat H ₄ – FLIPR	15 (10–22)	2.8 (2.6–3.1)
Mouse H ₄ – FLIPR	18 (15–23)	2.7 (2.0–3.5)
Rat H ₄ – GTPγS	60 (32–112)	66 (64–68)
Mouse mast cells – shape change	316 (210–476)	594 (336–1054)

^a*n* ≥ 3.^b95% confidence interval.

FLIPR, fluorometric imaging plate reader.

**Figure 2** Representative dose–response curves for inhibition of histamine-induced shape change of mouse bone marrow-derived mast cells by A-940894 and JNJ777120. Changes in shape change were determined as described in the *Methods* and are presented as % shape change. Data represent the mean ± SEM for each concentration and are representative of three individual experiments.*Functional antagonism at native histamine H₄ receptors in vitro*

The ability of A-940894 to inhibit native rodent and human H₄ receptor-mediated responses in immune cells was also evaluated. First, we established a histamine-induced shape change using mouse bone marrow-derived mast cells. In these experiments, histamine-induced shape change in a dose-dependent manner giving an average EC₅₀ value of 3.7 μmol·L⁻¹ (pEC₅₀ = 5.42 ± 0.01, *n* = 7). A-940894 blocked this response in a concentration-dependent manner with a resultant IC₅₀ value of 1.3 ± 0.1 μmol·L⁻¹ (K_b value of 316 nmol·L⁻¹, see Table 2) and complete blockade at 10 μmol·L⁻¹ (Figure 2). Similarly, histamine response was blocked by JNJ777120 with a mean IC₅₀ value of 4.0 ± 1.0 μmol·L⁻¹ (K_b = 594 nmol·L⁻¹, Table 2, Figure 2). A-940894 also behaved as an antagonist at the native human H₄ receptors, inhibiting chemotaxis of human eosinophils towards 1 μmol·L⁻¹ histamine (Figure 3). JNJ777120 behaved as an antagonist in this assay as well, displaying dose-dependent inhibition of histamine-stimulated chemotaxis (Figure 3), in good agreement with the previously reported data (Ling *et al.*, 2004). Chemotaxis of purified eosinophils towards different concentrations of histamine in these experiments was observed with a mean EC₅₀ value of 36 nmol·L⁻¹ (pEC₅₀ = 7.54

**Figure 3** Representative dose–response curves for inhibition of histamine-evoked eosinophil chemotaxis by A-940894 and JNJ777120. Chemotaxis experiments were performed as described in the *Methods*. Cell migration was analysed in quintuplicate for each concentration point. Data represent the means ± SEM of the percent control response (stimulation with 1 μmol·L⁻¹ histamine) and from one of two individual experiments.**Table 3** Pharmacokinetic properties of A-940894

Species	Route of administration and dose (mg·kg ⁻¹)	t _{1/2} (h)	C _{max} (μmol·L ⁻¹)	V _β (L·kg ⁻¹)	F (%)
Mouse	i.v. (5)	4.9	0.71	88.9	–
	p.o. (10)	6.8	0.13	–	33
	s.c. (10)	3.3	2.52	–	–
Rat	i.v. (10)	2.2	4.16	20	–
	p.o. (10)	3.4	0.51	–	37
	i.p. (10)	3.1	1.63	–	78

± 0.13, *n* = 6), in line with data previously reported by Ling *et al.* (2004).

Pharmacokinetics

The pharmacokinetic parameters for A-940894 are summarized in Table 3. A-940894 had a range of t_{1/2} values across multiple administration routes in rats and mice (Table 3). Oral bioavailability for A-940894 after oral dosing was 37% and 33% in rat and mouse respectively, but considerably higher after i.p. dosing (10 mg·kg⁻¹) in rat. Following s.c. administration of A-940894 (10 mg·kg⁻¹) and JNJ777120 (20 mg·kg⁻¹), maximum plasma concentration (C_{max}) levels were 2.52 and 2.56 μmol·L⁻¹, respectively, in mouse and rat. Serum protein binding for A-940894 was 66% and 50.6% in rat and human respectively.

Zymosan-induced peritonitis

The relevance of the zymosan-induced peritonitis model to histamine H₄ receptor biology was first established by determining the levels of H₄ receptor in peritoneal lavage of wild-type mice. qRT-PCR analysis of peritoneal exudate cells showed that there was expression of H₄ receptor mRNA in cells derived from peritoneal lavage of naïve BALB/c. This level of expression was significantly lower in cells derived from WBB6F1/J-Kit^W/Kit^{W-v} (W/W^v mice) that are devoid of mast cells, whereas H₄ receptor expression levels in littermate

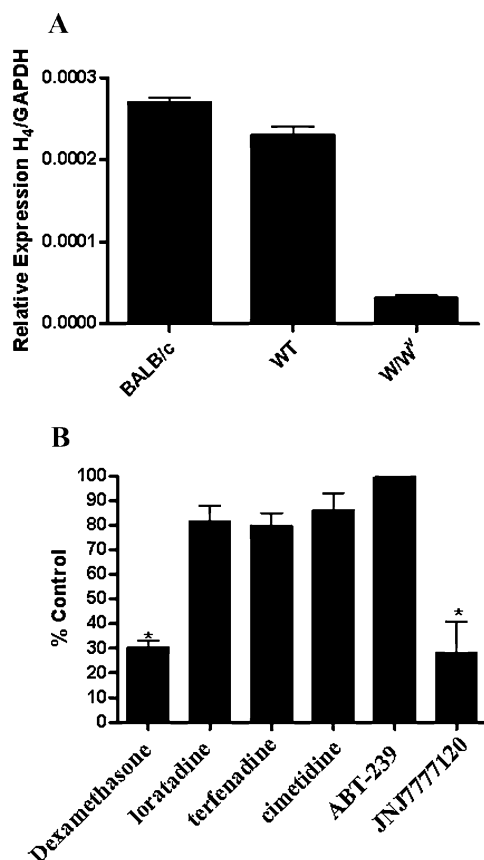


Figure 4 (A) Expression of histamine H₄ receptor mRNA in lavaged peritoneal cells from naïve BALB/c, wild-type WBB6F1/J-Kit^W and mast cell-deficient WBB6F1/J-Kit^W/Kit^{W^v} (W/W^v) mice. Levels of mRNA were determined as described in the *Methods* and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels for each mRNA sample. Data represent the mean \pm SEM for $n = 5$ animals for each group. (B) Effect of histamine receptor subtype antagonists on zymosan-induced neutrophil influx in BALB/c mice. Animals were given the drugs [H₁ antagonists, loratadine and terfenadine (30 mg·kg⁻¹ p.o.); H₂ antagonist, cimetidine (30 mg·kg⁻¹ p.o.); H₃ antagonist, ABT-239 (10 mg·kg⁻¹ i.p.); H₄ antagonist, JNJ777120 (30 mg·kg⁻¹ s.c.) and dexamethasone (10 mg·kg⁻¹ p.o.)] 30 min prior to zymosan (2 mg per animal). Lavage fluid was collected 4 h later and myeloperoxidase (MPO) activity measured as described in the *Methods*. Data represent the mean \pm SEM of the per cent control MPO activity for five to six animals for each group.

control WBB6F1/J-Kit^W wild type were similar to that found in BALB/c (Figure 4A). These data indicate that histamine H₄ receptors were associated predominantly with the mast cells in peritoneal lavage.

Intraperitoneal challenge with zymosan-induced leukocyte accumulation, predominantly of neutrophils (>80% of total leukocytes) in the mouse peritoneum, consistent with previous descriptions of the model (Doherty *et al.*, 1985). The presence of inflammatory mediators in the peritoneal lavage fluid was evaluated at 15, 30, 45, 90, 180, 240 and 360 min after zymosan administration (Figure 5). Both histamine and PGD₂ levels continued to increase in a time-dependent manner through 6 h time point, reaching values corresponding to 10- and 17-fold increases over basal levels (7.8 ng·mL⁻¹ for histamine and 3.8 ng·mL⁻¹ for PGD₂). Additionally, the increases in both histamine and PGD₂ appeared to occur in

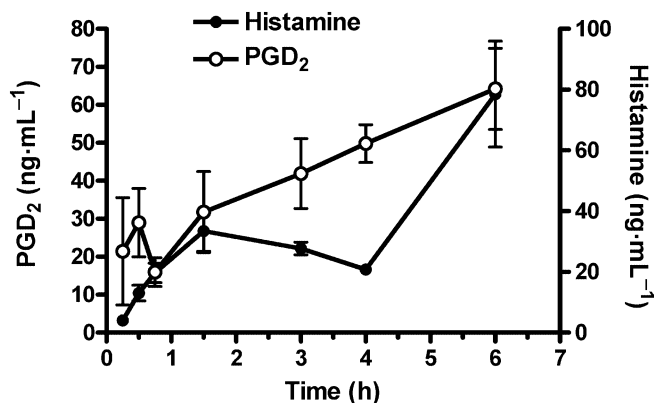


Figure 5 Time course for increases in histamine and prostaglandin D₂ (PGD₂) content of peritoneal lavage fluid obtained from mice given zymosan (2 mg per animal i.p.). Levels of histamine and PGD₂ were determined as described in *Methods*. Basal levels of histamine and PGD₂ were 7.8 ± 0.8 ng·mL⁻¹ and 3.8 ± 1.7 ng·mL⁻¹ respectively. Data represent the mean \pm SEM for five animals per time point.

two phases with the first phase peaking at 90 min for histamine and 30 min for PGD₂.

Profiling of the histamine receptor subtypes capable of mediating zymosan-induced neutrophil influx in BALB/c mice revealed that the H₁ receptor antagonists loratadine and terfenadine (30 mg·kg⁻¹ p.o.), the H₂ receptor antagonist cimetidine (30 mg·kg⁻¹ p.o.) and the H₃ receptor antagonist ABT-239 (10 mg·kg⁻¹ i.p.) had no effect on neutrophil influx (Figure 4B). Only the H₄ receptor antagonist JNJ777120 (30 mg·kg⁻¹ s.c.) significantly reversed neutrophil influx (72% inhibition), to levels comparable to those obtained with dexamethasone (10 mg·kg⁻¹ p.o.).

A-940894, given s.c., 30 min prior to injection of zymosan, resulted in an almost complete inhibition of neutrophil MPO activity in lavages collected 4 h after zymosan administration. With responses to dexamethasone set to 100% efficacy, A-940894 reduced MPO activity by 111%, 120% and 111% ($P < 0.01$) at 1, 3 and 10 mg·kg⁻¹ respectively (Figure 6A) relative to the dexamethasone control, while JNJ777120, at 10, 30 and 100 mg·kg⁻¹ s.c., showed significant inhibition of 88%, 98% and 86% ($P < 0.01$) (Figure 6B). PGD₂ levels were also determined in lavage fluid collected 1.5 h after zymosan administration. A-940894 dose-dependently reduced PGD₂ levels by 65% and 114% ($P < 0.01$) at 3 and 10 mg·kg⁻¹, relative to the dexamethasone control, while no reduction was observed at 1 mg·kg⁻¹ (Figure 6C). JNJ777120, given at 10, 30 and 100 mg·kg⁻¹ s.c., significantly decreased PGD₂ levels at all doses tested by 87%, 91% and 936% ($P < 0.01$) respectively (Figure 6D). Similar results were observed for PGE₂ levels (data not shown). This inhibition was selective for PGs, as no alteration in levels of other inflammatory mediators, including cysteinyl leukotrienes, leukotriene B₄ or the chemokine CXCL1, was observed (data not shown). A selective reduction in PGD₂ was also observed in the mast cell-deficient W/W^v mice.

Discussion and conclusions

The newly identified, non-imidazole histamine H₄ receptor antagonist A-940894 was found to bind with high affinity to

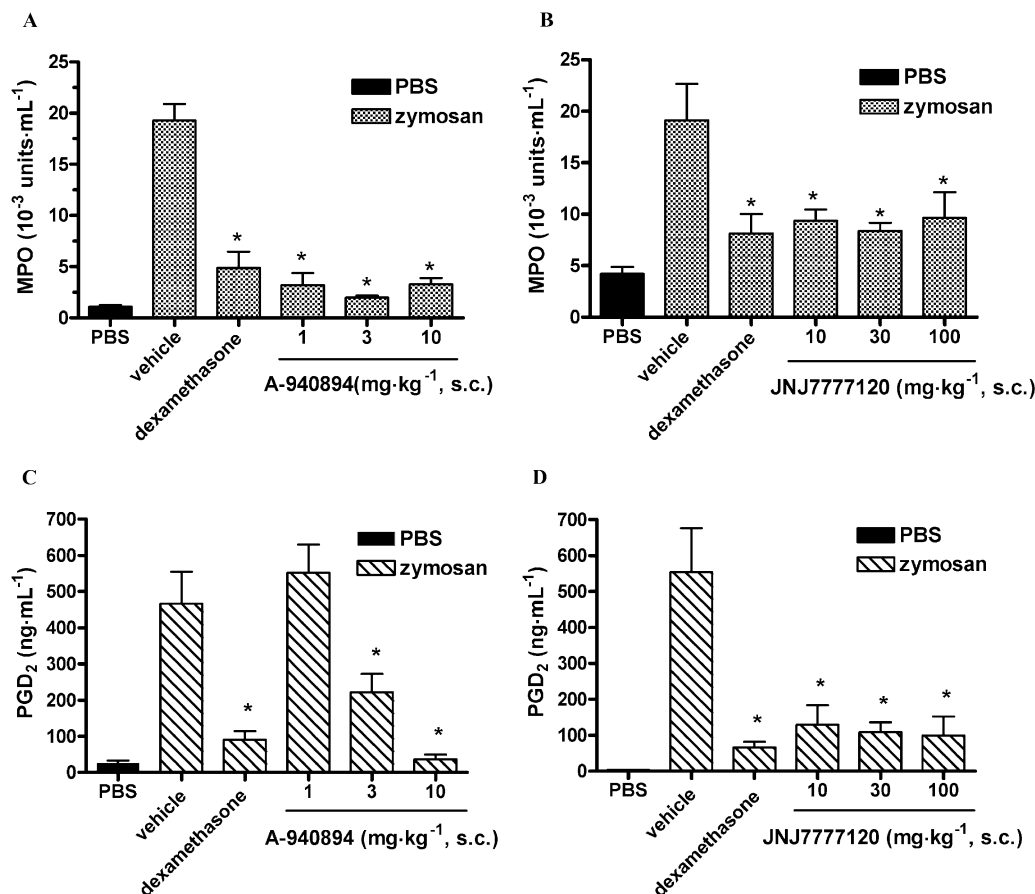


Figure 6 Inhibitory effects of increasing i.p. doses of A-940894 and JNJ777120 on zymosan-induced, mouse peritoneal neutrophil myeloperoxidase (MPO) activity and prostaglandin D₂ (PGD₂) levels in mice. MPO activity (units·mL⁻¹ lavage) and levels of PGD₂ (ng·mL⁻¹) were determined as described in *Methods*. Data represent the means \pm SEM for five animals per group. Significant differences compared with vehicle group are indicated as * $P < 0.05$.

rat and human recombinant H₄ receptors and is over 50-fold selective for the human histamine H₄ receptor versus other histamine receptor subtypes, an *in vitro* profile similar to previously described histamine H₄ receptor antagonists A-943931, A-987306 and JNJ777120 (Jablonowski *et al.*, 2003; Cowart *et al.*, 2008; Liu *et al.*, 2008). A broad selectivity binding screen at high concentration of the compound revealed interactions with several additional binding sites (e.g. β_1 -adrenoceptor, M₄ muscarinic, μ opioid, 5-HT_{1A}, 5-HT_{1B}, 5-HT₃ receptors and the 5-HT transporter). However, because these sites are not usually associated with inflammatory responses discussed in this report, their involvement in the A-940894-induced responses is unlikely. In cells co-expressing the chimeric G α_{q15} protein with human, rat or mouse histamine H₄ receptors, A-940894 potently and dose-dependently blocked histamine-evoked intracellular calcium responses. Comparable potencies at the rat and mouse H₄ receptors (15–18 nmol·L⁻¹) and somewhat lower potency (74 nmol·L⁻¹) for the human receptor were demonstrated, reflecting the 10-fold lower binding affinity of A-940894 for the human H₄ receptor when compared with the rat receptor. Pharmacological species differences have been observed for other histamine H₄ receptor ligands, including histamine itself (Liu *et al.*, 2001b). A-940894 also displayed potent antagonism in [³⁵S]-GTP γ S

binding in cell membranes expressing rat H₄ receptors, dose-dependently inhibiting histamine-induced [³⁵S]-GTP γ S binding ($K_b = 60$ nmol·L⁻¹). Interestingly, in the absence of histamine, this compound did not affect basal GTP γ S binding activity (data not shown); thus it is not an H₄ receptor inverse agonist in this assay, nor a partial agonist at the rat H₄ receptor.

We further characterized the ability of A-940894 to antagonize histamine-induced responses using native H₄ receptor systems. A-940894 was able to inhibit both histamine-mediated shape change of the bone marrow-derived mouse mast cells and histamine-induced chemotaxis of purified human eosinophils. Interestingly, in the shape change assay with mouse mast cells, A-940894 displayed *in vitro* potencies that were ~17-fold lower than those determined in the mouse recombinant H₄ receptor-mediated intracellular calcium assay. The prototypic H₄ receptor antagonist JNJ777120 (Jablonowski *et al.*, 2003) also exhibits substantially lower potency in antagonizing native histamine H₄ receptor responses in comparison with recombinant receptors. Whether these lower potencies seen at the native H₄ receptor are reflective of differences in signalling responses between the native and recombinant receptors, conformational differences between the receptors, or other factors, remains to be

determined. Mast cells are known to be one of the main sources of histamine in the body, so it is conceivable that continuous exposure of H₄ receptors to histamine over the period of time that is required for generation of mature mast cells in culture could lead to H₄ receptor desensitization. Nevertheless, A-940894 effectively blocks H₄ receptor-mediated immune cell responses in a concentration-dependent manner *in vitro*, including mouse mast cell shape change and human eosinophil chemotactic responses. These findings are consistent with the known expression of histamine H₄ receptors in differentiated mast cells derived from mouse bone marrow (Hofstra *et al.*, 2003) and in human eosinophils (Buckland *et al.*, 2003; Ling *et al.*, 2004) as well as the findings that JNJ777120 inhibits calcium mobilization and chemotaxis in these cells (Hofstra *et al.*, 2003; Ling *et al.*, 2004; Thurmond *et al.*, 2004). A variety of inflammatory disease states, such as asthma and inflammatory bowel disease, are linked to infiltration of eosinophils; thus blockade of this response via H₄ receptor antagonism may offer a novel means of treating these ailments (Varga *et al.*, 2005; Dunford *et al.*, 2006). Overall, the *in vitro* findings demonstrate that A-940894 is a potent and selective histamine H₄ receptor antagonist at recombinant human, rat and mouse H₄ receptors as well as at native histamine H₄ receptors expressed in mouse mast cells and human eosinophils.

A commonly used model of acute inflammation is the mouse model of zymosan-induced peritonitis that culminates in peritoneal neutrophil influx (Doherty *et al.*, 1985; Ajuebor *et al.*, 1998; Ajuebor *et al.*, 1999). This model has previously been shown to be both mast cell- (Kolaczowska *et al.*, 2001a) and histamine H₄ receptor-dependent (Thurmond *et al.*, 2004). Further characterization of this model in our laboratories revealed that along with an increase in peritoneal neutrophils upon exposure to zymosan, a concomitant increase in inflammatory mediators including histamine and PGD₂ was also seen. Basal levels of histamine increased 10-fold from 7.8 to 80 ng·mL⁻¹ over a 6 h time course in a time-dependent manner while PGD₂ levels increased 17-fold from 3.7 to over 60 ng·mL⁻¹. Interestingly, the increases in these inflammatory mediators appeared to be biphasic. Histamine showed an initial early spike in levels at 1.5 h after zymosan administration followed by a decrease in levels to 4 h and another large increase by 6 h. In contrast, PGD₂ levels showed an earlier initial spike in levels, by 30 min following zymosan administration, a decline in levels at 45 min, followed by a steady increase in PGD₂ levels up to 6 h. Whether these time-dependent fluctuations in the levels of these inflammatory mediators reflect the time-dependent recruitment and activation of mast cells, neutrophils or other immune cells and the subsequent release from these cells remains to be determined. Additional evidence supporting the critical role of the histamine H₄ receptor in mediating mast cell-dependent inflammatory events was provided by the finding that the level of H₄ receptor mRNA obtained from lavaged peritoneal cells from naïve animals was more than sevenfold lower in mast cell-deficient W/W^v mice when compared with their normal WBB6F1/J-Kit^W controls or with BALB/c mice. This suggests that resident mast cells may be the predominant H₄ receptor-expressing cell type in the peritoneum that is responsible for mediating inflammatory reactions to substances such as

zymosan. Additionally, further characterization of the histamine receptor subtypes responsible for mediating zymosan-induced influx of neutrophils into the peritoneum confirmed previous findings that the H₄ receptor mediated this response. Our results also demonstrated that the H₄ receptor was the only histamine receptor subtype involved as antagonists of H₁, H₂ and H₃ receptors did not block this inflammatory response.

We have shown here that in addition to the direct inhibitory effect on H₄-mediated mast cell effects *in vitro*, the H₄ antagonist A-940894 has potent *in vivo* anti-inflammatory effects, completely blocking neutrophil influx in the mouse zymosan-induced peritonitis model at doses as low as 1 mg·kg⁻¹ s.c. Interestingly, A-940894 also diminished PGD₂ levels in a dose-dependent fashion with complete reversal of the increased levels at 10 mg·kg⁻¹, although no inhibition of PGD₂ responses was observed at 1 mg·kg⁻¹. A-940894 also inhibited increases in PGE₂ levels that were seen in this model (data not shown), but had no effect on leukotrienes or inflammatory chemokine CXCL1, suggesting that H₄ receptors may play a role in regulating PG production in mast cells. This hypothesis is further supported by the finding that PGD₂ levels are reduced in mast cell-deficient mice. Similarly, JNJ777120 blocked peritoneal neutrophil influx induced by zymosan at 10 mg·kg⁻¹ (as has been previously demonstrated by Thurmond *et al.*, 2004) and also blocked the increases in PGD₂ levels. The inhibition of PGD₂ by both A-940894 and JNJ777120 suggests that histamine H₄ receptors may play a role in regulating PG production in mast cells. However, as A-940894 appears to be 3–10 times less potent as an inhibitor of PGD₂ output, compared with its inhibition of neutrophil influx, it is also possible that this effect is due to secondary pharmacology of the antagonists towards an unknown target. The favourable pharmacokinetic properties of A-940894 across multiple routes of administration in mouse and rat (*t*_{1/2} from 2.2 to 6.8 h in rat and mouse dosed p.o. good bioavailability) predicted that this compound would be useful *in vivo* for determining the H₄ receptor-dependent anti-inflammatory properties of this compound. The longer half-life of A-940894 in rats and especially mice, a commonly used animal model of inflammation, in comparison with other H₄ receptor antagonists such as JNJ777120 (mouse *t*_{1/2} = 0.5–1.4 h, data not shown), suggests that this compound would be potentially more useful to determine the effects of long-term blockade of H₄ receptors by chronic dosing in inflammatory disease states than previously reported H₄ receptor antagonists.

Our current study as well as previously reported findings (Ajuebor *et al.*, 1999; Kolaczowska *et al.*, 2001a,b; Hofstra *et al.*, 2003; Thurmond *et al.*, 2004; Godot *et al.*, 2007) strongly suggest several common participants with respect to the zymosan-induced peritonitis model of inflammation. These include mast cells, the H₄ receptor, histamine and other inflammatory mediators. It has been hypothesized, and findings to date seem to indicate, that histamine H₄ receptors expressed on mast cells may regulate mast cell chemotaxis as well the synthesis and release of pro-inflammatory mediators, including PGs, chemoattractants (i.e. leukotriene B₄) and possibly other mediators (chemokines and cytokines). These mediators can then modulate the activity of other local

immune cells, such as macrophages, as well as recruit infiltrating cell types, such as neutrophils and eosinophils, to further mediate the inflammatory response. The findings that H₄ receptor antagonists such as A-940894 can inhibit immune cell chemotaxis *in vitro* and can reduce inflammatory responses *in vivo* by decreasing the influx of neutrophils and local levels of inflammatory mediators such as PGD₂ following an inflammatory response suggest that the H₄ receptor plays an important role in mediating inflammatory processes.

In conclusion, we have demonstrated that A-940894 is a potent histamine H₄ receptor antagonist with at least a 50-fold selectivity over other histamine receptor subtypes. Additionally, A-940894 blocks the activation of histamine-stimulated effects in cells involved in inflammatory processes, including H₄ receptor-mediated shape change of mouse bone marrow-derived mast cells as well as human eosinophil chemotaxis. This compound also exhibits potent *in vivo* anti-inflammatory activity, including antagonism of H₄ receptor-mediated zymosan-induced peritonitis and concomitant inhibition of increases in the intraperitoneal levels of the inflammatory mediator PGD₂. A-940894 exhibits favourable pharmacokinetic properties with a longer half-life in mouse than that for other described H₄ receptor antagonists that should allow coverage to determine the role of H₄ receptors in chronic inflammation models. Together with other H₄ receptor antagonists, A-940894 will serve as a useful tool compound to further investigate the role of histamine H₄ receptors in inflammatory responses and the potential utility of this target for drug treatment of human inflammatory disease states.

Statement of conflict of interest

All authors are employees of Abbott Laboratories, which funded the research.

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