Supplemental material to this article can be found at:
http://jpet.aspetjournals.org/content/suppl/2014/02/18/jpet.113.211714.DC1

1521-0103/349/2/176–184$25.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 2014 by The American Society for Pharmacology and Experimental Therapeutics
http://dx.doi.org/10.1124/jpet.113.211714
J Pharmacol Exp Ther 349:176–184, May 2014

Clinical and Preclinical Characterization of the Histamine H_4 Receptor Antagonist JNJ-39758979[S]

Robin L. Thurmond, Bin Chen, Paul J. Dunford, Andrew J. Greenspan, Lars Karlsson,[1] David La, Peter Ward, and Xie L. Xu

Janssen Research & Development, L.L.C., San Diego, California

Received December 2, 2013; accepted February 14, 2014

ABSTRACT

The histamine H_4 receptor (H_4R) has been shown to have preclinical involvement in both inflammatory and pruritic responses. JNJ-39758979 [(*R*)-4-(3-amino-pyrrolidin-1-yl)-6-isopropyl-pyrimidin-2-ylamine] is a potent and selective H_4R antagonist with a K_i at the human receptor of 12.5 ± 2.6 nM and greater than 80-fold selectivity over other histamine receptors. The compound also exhibited excellent selectivity versus other targets. JNJ-39758979 showed dose-dependent activity in models of asthma and dermatitis consistent with other H_4R antagonists. Preclinical toxicity studies of up to 6 months in rats and 9 months in monkeys indicated an excellent safety profile, supporting the clinical testing of the compound. An oral formulation of JNJ-39758979 was studied in a phase 1 human volunteer study to assess safety, pharmacokinetics, and pharmacodynamics. The compound was well tolerated, with the exception of dose-dependent nausea, and no safety issues were noted in the phase 1 study. JNJ-39758979 exhibited good pharmacokinetics upon oral dosing with a plasma half-life of 124–157 hours after a single oral dose. In addition, dose-dependent inhibition of histamine-induced eosinophil shape change was detected, suggesting that the H_4R was inhibited in vivo. In conclusion, JNJ-39758979 is a potent and selective H_4R antagonist that exhibited good preclinical and phase 1 safety in healthy volunteers with evidence of a pharmacodynamics effect in humans.

Introduction

The histamine H_4 receptor (H_4R) has attracted interest as a potential drug target since its discovery in 2000. Preclinical data suggest a role for the H_4R in a variety of inflammatory diseases. Antagonists of the receptor reduce inflammation and improve lung function in mouse and guinea pig asthma models (Dunford et al., 2006; Cowden et al., 2010a; Somma et al., 2013). Efficacy has also been shown in mouse dermatitis models and a rat model of colitis (Varga et al., 2005; Cowden et al., 2010b; Suwa et al., 2011; Matsushita et al., 2012). Recently, anti-inflammatory activity in mouse arthritis models has been reported with H_4R antagonists (Nent et al., 2013; Cowden et al., 2014). Mice deficient in the H_4R are protected in the asthma, dermatitis, and arthritis models, further boosting the conclusion that inhibiting the receptor would yield anti-inflammatory effects in humans (Dunford et al., 2006; Cowden et al., 2010b, 2014). However, the H_4R may not play the same role in all diseases, as it appears that neuronal inflammation in experimental autoimmune encephalomyelitis models is exacerbated in H_4R-deficient mice or with treatment with an H_4R antagonist (del Rio et al., 2012; Ballerini et al., 2013). In addition to a role in inflammation, the receptor also appears to control pruritus in numerous preclinical models of itch (Dunford et al., 2007; Yamaura et al., 2009; Rossbach et al., 2011; Ohsawa and Hirasawa, 2012; Shin et al., 2012). These data, along with the anti-inflammatory data in dermatitis models, suggest that H_4R antagonists may be useful in the treatment of atopic dermatitis.

Preclinical data with H_4R receptor antagonists have generated interest in exploring the use of such antagonists in humans. To date, limited clinical data on H_4R antagonists has been reported. Data from phase 1 studies with two H_4R antagonists, UR-63325 and PF-3893787 [(*R*)-*N*4-(cyclopropylmethyl)-6-(3-(methylamino)pyrrolidin-1-yl)pyrimidine-2,4-diamine], have been reported at scientific meetings and are summarized in a review by Salcedo et al. (Salcedo et al., 2013). UR-63325 was reported to have been studied in both single dose and multiple doses in healthy volunteers with no significant safety issues. The compound was also shown to have a pharmacodynamic effect in inhibiting the histamine-induced shape change in eosinophils ex vivo. Similar findings were reported with PF-3893787 another H_4R antagonist (Mowbray et al., 2011). In the current work the detailed phase 1 clinical safety, pharmacokinetic, and pharmacodynamics data with the selective H_4R antagonist JNJ-39758979 are presented.

[1]Current Address: Novo Nordisk A/S, Måløv, Denmark.
dx.doi.org/10.1124/jpet.113.211714.
[S] This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: $AUC_{0\text{-}24h}$, area under the curve from time 0 to 24 h; AUC_{last}, area under the curve from time 0 to the last measured time point; GLP, good laboratory practice; H_4R, histamine H_4 receptor; JNJ-39758979, (*R*)-4-(3-amino-pyrrolidin-1-yl)-6-isopropyl-pyrimidin-2-ylamine; MAD, multiple ascending dose; NOAEL, no observed adverse effect level; PF-3893787, (*R*)-*N*4-(cyclopropylmethyl)-6-(3-(methylamino)pyrrolidin-1-yl) pyrimidine-2,4-diamine; PK, pharmacokinetic; SAD, single ascending dose; T_{max}, time at which maximum plasma concentration is observed; V_d/F, volume of distribution.

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

Materials and Methods

JNJ-39758979 [(*R*)-4-(3-amino-pyrrolidin-1-yl)-6-isopropyl-pyrimidin-2-ylamine](Fig. 1) was synthesized as previously described (Savall et al., 2014).

Pharmacology. Binding and functional assays for the various histamine receptors were carried out as described previously (Thurmond et al., 2004; Yu et al., 2010). Muscarinic receptor binding assays were carried out with cell membranes from Chinese hamster ovary cells transfected with human M_1, M_2, M_3, and M_4 receptors. The radioligand used was 0.2 nM [^{3}H]*N*-methyl scopolamine, and nonspecific binding was defined with 1 μM unlabeled atropine. A panel of 50 different biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and neurotransmitter transporter binding assays was run by Cerep, Inc. (Redmond, WA). The full methods and references can be found on the Cerep website (www.cerep.fr). The assays were run at 1 μM JNJ-39758979. The kinase selectivity was determined with the KinaseProfiler panel (Gao et al., 2013) and was run by EMD Millipore Corp. (San Diego, CA). Histamine-induced eosinophil shape change was conducted as previously described (Ling et al., 2004; Yu et al., 2010). For the whole-blood assay, one set of samples was untreated and not manipulated before measurement. Others were processed as previously described, but in the absence of histamine (baseline), as the processing itself leads to some level of eosinophil activation. The final set of samples were processed and treated with histamine with or without JNJ-39758979. Statistical analysis for the eosinophil shape-change data were carried out with a Student's *t* test for all statistical comparisons between two groups and a one-way analysis of variance with post-hoc Dunnett's test for comparison of three or more groups. The ovalbumin mouse asthma model and the fluorescein isothiocyanate-induced mouse dermatitis model were conducted as previously described (Dunford et al., 2006; Cowden et al., 2010b). JNJ-39758979 was dosed orally in 20% hydroxypropyl-$\beta-$cyclodextrin. For the asthma model the compound was administered 20 minutes prior to the daily allergen challenge. In the fluorescein isothiocyanate model the compound was given 30 minutes before and 4 hours after fluorescein isothiocyanate application to the ear. Statistical analysis was carried out with a one-way analysis of variance with post-hoc Dunnett's test.

Toxicology Studies. JNJ-39758979 was evaluated in repeat-dose toxicity studies for up to 6 months duration in Sprague-Dawley rats and 9 months in cynomolgus monkeys. Studies in rats were conducted with the following doses and study duration: 0, 10, 50, and 300 mg/kg per day (n = 10/sex/group) for 1 month; 0, 10, 50, and 250 mg/kg per day (n = 10/sex/group) for 3 months; and 0, 25, 50, 100, and 200 mg/kg per day (n = 20/sex/group) for 6 months. Studies in monkeys were conducted with the following doses and study duration: 0, 6, 20, and 60 mg/kg per day (n = 3/sex/group) for 1 month; 0, 2, 6, and 30 mg/kg per day (n = 4/sex/group) for 3 months; and 0, 3, 10, and 30 mg/kg per day (n = 4/sex/group) for 9 months. JNJ-39758979 was formulated in water and administered daily by gavage. Rats and monkeys were examined for mortality, clinical signs, ophthalmoscopic changes, body weight, food consumption, hematology, clinical chemistry, anatomic pathology, and toxicokinetics. In addition, monkeys were examined for electrocardiographic changes. Rats and monkeys were also assessed for reversal of any effects following a 1-month recovery period. These studies were conducted in compliance with good laboratory practice (GLP) regulations.

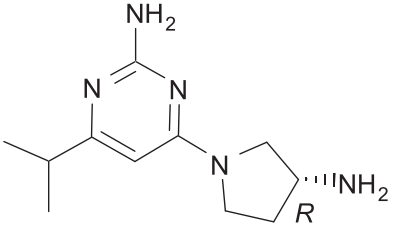

Fig. 1. The structure of JNJ-39758979.

Clinical Study. This was a single-center, double-blind, randomized, placebo-controlled study in healthy male and female subjects of nonchildbearing potential (postmenopausal or otherwise sterile). The study was conducted in Belgium from September 2008 to June 2009. JNJ-39758979 was supplied as a powder for reconstitution with sterile water to provide oral solutions of 5 and 100 mg/ml. In the single ascending dose (SAD) portion of the study, eligible male subjects in each treatment group were randomized to receive either a single oral dose of JNJ-39758979 (n = 6) or placebo (n = 3) after an overnight fast. Dose levels for the different groups were 10, 30 (n = 5 only), 100, 300, 600, and 1200 mg of JNJ-39758979. The doses of JNJ-39758979 were escalated in a stepwise fashion if the safety, tolerability, and plasma pharmacokinetic profile (up to 24 hours) were deemed acceptable. In the multiple ascending dose (MAD) part of the study, male subjects received oral doses of placebo or 30, 100 (n = 5 only), 300, or 600 mg (300 mg twice daily) of JNJ-39758979 for 14 consecutive days. The doses were escalated in a stepwise fashion if the safety, tolerability, and plasma pharmacokinetic profile were deemed acceptable. In addition female subjects were dosed with either 300 mg of JNJ-39758979 (n = 6) or placebo (n = 3) for 14 consecutive days. There were no early withdraws in the SAD part of the study; however, in the MAD portion of the study two placebo patients discontinued early. The main criteria for inclusion in the study were healthy male or postmenopausal or nonchildbearing (i.e., surgically sterile) female subjects between 18 and 55 years of age, inclusive; body mass index within 18–29 kg/m^2, with a minimum body weight of 50 kg; and supine blood pressure (after resting for 5 minutes) between 90–139 mm Hg systolic and 50–89 mm Hg diastolic, inclusive. For all parts of the study, adverse events and concomitant medications were assessed and recorded from screening through follow-up. The following safety measures were assessed at various time points during the study: medical history, physical examination, neurologic examination, 12-lead electrocardiogram (ECG), continuous ECG monitoring (telemetry), and vital signs (blood pressure, heart rate, respiratory rate, and temperature). Safety measures also included clinical laboratory tests: blood chemistry; hematology, coagulation, and serology tests (hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antibody); urinalysis; alcohol analysis; urine pregnancy test and serum pregnancy test (females); urine drug screen; 24-hour urine for creatinine clearance, protein, and albumin excretion rate; and spot urine albumin/creatinine ratio.

Pharmacokinetic Evaluation. For all parts of the clinical study, venous blood samples were taken for the measurement of JNJ-39758979 plasma concentrations. A validated, specific and sensitive method for a protein extraction procedure with liquid chromatography coupled to tandem mass spectrometry was developed for analysis of plasma samples with K_2EDTA anticoagulant to determine concentrations of JNJ-39758979 over a range of 2 to 500 ng/ml. The analytical reference standard of JNJ-39758979 and an internal standard were used to quantitate JNJ-39758979 in these plasma samples. Pharmacokinetic parameters were determined from plasma data of JNJ-39758979 after single oral administration or after multiple oral administrations. Data obtained from placebo subjects was not included in the pharmacokinetic (PK) analysis, as all plasma concentration data obtained for these subjects were below the lower limit of quantification (2 ng/ml). Because of insufficient numbers of time points with concentrations above the lower limit of quantification in some cohorts of the SAD portion of the study, only the maximum concentration (C_{max}) and time of maximum concentration (T_{max}) were reported for subjects in the 10-mg treatment group, whereas C_{max}, T_{max}, area under the curve from time 0 to 24 hours (AUC_{0-24h}), and the area under the curve from time 0 to the last measured time point (AUC_{last}) were reported for the 30-mg treatment group.

Pharmacodynamic Assay. Venous blood samples were collected into potassium EDTA tubes at the time points specified. The collected blood volumes were 4.9 ml. To measure histamine-induced eosinophil shape change, 1 ml of blood was treated with 0 or 0.3 μM histamine in the presence of 3 μM ranitidine for 10 minutes at 37°C. Each

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

treatment was done in duplicate. Following the histamine stimulation, the blood was fixed and the shape change of eosinophils was measured by gated autofluorescence forward scatter assay on a Bayer Advia 120 clinical hematology analyzer (Erlangen, Germany). Two scans were acquired for each replicate. Repeated scans were summarized by calculating the average of the means of the forward scatter. Sample replicates were summarized by calculating the average of the scan replicates. The eosinophil population was gated by high peroxidase staining and low forward scatter, based on positive and negative controls provided by the Advia 120 manufacturer. To evaluate the effect of compound on eosinophil forward scatter upon histamine stimulation, the variable "% Change" was calculated as: (Mean Scatter_h − Mean Scatter_b) / Mean Scatter_b × 100, where Mean Scatter_h denotes the mean scatter of eosinophils treated with 0.3 μM histamine, and Mean Scatter_b denotes the mean scatter of eosinophils treated with control (no histamine). Statistical analysis was carried out using a one-way analysis of variance with post-hoc Dunnett's test.

In addition to histamine-induced eosinophil shape change, serum was collected in the MAD portion of the study predose on days 1, 2, 7, and 14, as well as 4-hours post-dose on day 1. Serum markers were measured with a multiplex enzyme-linked immunosorbent assay: TruCulture MAP, Antigen Map v1.6 (Rules-Based Medicine, Austin, TX). MAP assays were performed at Rules-Based Medicine (Austin, TX). For each assay in Rules-Based Medicine MAP, values below the detection limit were replaced with the lowest value in the given assay, or the least detectable dose of the assay, whichever is smaller. The least detectable dose was determined as the mean plus 3 times the standard deviation of 20 blank readings.

Results

In Vitro Pharmacology. The pharmacological activity of JNJ-39758979 at the histamine H_1, H_2, H_3, and H_4 receptors of various species was investigated in vitro. JNJ-39758979 has a high affinity for the human H_4R with a K_i value of 12.5 ± 2.6 nM (Table 1). The compound did not display agonist activity in the systems tested up to concentrations of 10 μM and in fact displayed properties of a competitive antagonist of histamine with a pA_2 value of 7.9 ± 0.1. JNJ-39758979 exhibits high affinity for the mouse and monkey H_4R with less affinity for the rat, guinea pig, and dog receptors. The compound was a competitive antagonist at the mouse, rat, and monkey receptors with no agonist activity detected. The compound is a weak ligand for the human H_3 receptor and has modest affinity for the mouse and rat H_3 receptor. For the human and mouse, there is a good separation between H_4R and H_3 receptor affinity; however, for the rat the affinity is similar. There is little, if any, affinity for the H_1 and H_2 receptors.

JNJ-39758979 was evaluated for selectivity against other nonhistamine receptor targets. These targets represent major classes of biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and neurotransmitter transporters. There was less than 20% inhibition at 1 μM for all of the targets, except for a 64% inhibition detected for the muscarinic M_3 receptor (Supplemental Table S1). Since this initial assay was a screening assay, the inhibition of the human muscarinic receptors was followed up by more definitive detailed determination of the K_i values in transfected cells. JNJ-39758979 had no affinity up to 10 μM against the human muscarinic M_1, M_2, M_3, or M_4 receptors. The difference in M_3 results between the inhibition assay and the K_i determination assay is most likely accounted for by the single concentration analyzed in the inhibition assay and the fact that this is a high-throughput format. JNJ-39758979 was also tested at 10 μM against 66 kinases and did not show greater than 25% inhibition for any of the kinases tested (Supplemental Table S2).

TABLE 1
K_i, EC_{50}, and pA_2 values for JNJ-39758979 at histamine receptors from various species

Receptor	Species	K_i[a]	EC_{50}	pA_2[a]
		nM	*μM*	
H_4	Human	12.5 ± 2.6	>10	7.9 ± 0.1
H_4	Mouse	5.3 ± 0.4	>10	8.3 ± 0.2
H_4	Rat	188 ± 61	>10	7.2 ± 0.2
H_4	Monkey	25 ± 1	>10	7.5 ± 0.1
H_4	Guinea pig	306 ± 23	ND	ND
H_4	Dog	>10,000	ND	ND
H_3	Human	1043 ± 348	ND	ND
H_3	Mouse	202 ± 29	ND	ND
H_3	Rat	258 ± 26	ND	ND
H_2	Human	>1000	ND	ND
H_1	Human	>1000	ND	ND
H_1	Mouse	6800 ± 1800	ND	ND
H_1	Monkey	>10,000	ND	ND

ND, not determined.
[a]Data given as ± S.E.M. if the assay was run at least twice.

The pharmacologic activity of JNJ-39758979 on the human eosinophil histamine-induced shape change was investigated in vitro. Histamine is able to induce chemotaxis of eosinophils and this can be represented by a change in cell shape (Ling et al., 2004). Increasing concentrations of JNJ-39758979 caused a rightward shift in the histamine dose-response for inducing eosinophil shape change, indicating that the compound was behaving as an antagonist (Fig. 2A). The assay cannot be conducted under equilibrium conditions since the reaction occurs within minutes of adding the agonist; therefore, it is not possible to derive meaningful IC_{50} or K_i values (Ling et al., 2004). When tested in whole blood, JNJ-39758979 caused a dose-dependent inhibition in histamine-induced eosinophil shape change (Fig. 2B). In both cases, significant inhibition of eosinophil shape change was observed at concentrations equal to or greater than 100 nM (22 ng/ml). JNJ-39758979 also inhibited the histamine-induced mouse bone marrow–derived mast cell chemotaxis with an IC_{50} of 8 nM when 10 μM histamine was used. An estimation of the K_i can be made using the Cheng-Prusoff equation given an EC_{50} for histamine of 10 μM. This yields an apparent K_i of 4 nM, consistent with the results from the mouse-binding assay (5 nM).

In Vivo Pharmacology. Previously, it had been shown that the H_4R mediates inflammation in a murine asthma model (Dunford et al., 2006), and therefore, effects of JNJ-39758979 were investigated in this model. In ovalbumin-sensitized mice, different doses of JNJ-39758979 were administered orally before each of the daily challenges of ovalbumin. Under these conditions, JNJ-39758979 showed dose-related inhibition of cellular inflammation induced by ovalbumin challenge compared with vehicle-treated animals (Fig. 3A). A significant reduction of eosinophils in lavage fluid was observed at 0.2 mg/kg doses and higher compared with vehicle treatment animals. There was no statistically significant difference between any of the dose groups. There was a trend for inhibition of total cells and lymphocytes, but this did not reach statistical significance. No changes in macrophages or neutrophils were observed. The effect of JNJ 7777120 is shown for comparison and was similar to that previously reported (Dunford et al., 2006). The C_{max} of the compound at 0.2 mg/kg based on previously published

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

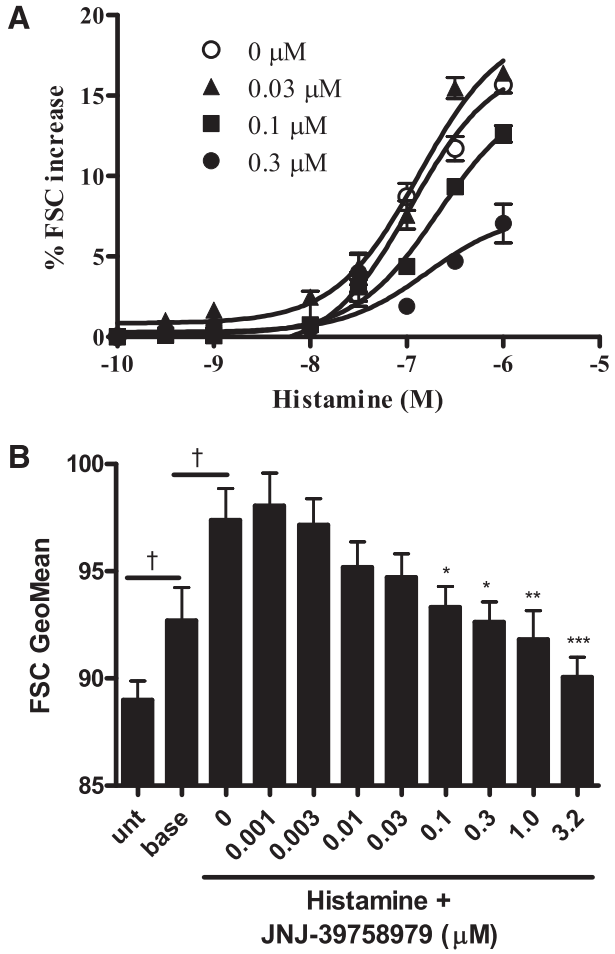

Fig. 2. The effects of JNJ-39758979 on histamine-induced eosinophil shape change. (A) Human polymorphonuclear leukocytes were stimulated with various concentrations of histamine in addition to 0 μM (○), 0.03 μM (▲), 0.1 μM (■), or 0.3 μM (●) JNJ-39758979. After 5 minutes the percent increase in forward scatter was measured by flow cytometry. The mean (n = 2) and S.E.M. are given. (B) Human blood was incubated with various concentrations of JNJ-39758979 prior to stimulation with 300 nM histamine. The geometric mean (four replicates from four different donors) of the FSC GeoMean was measured by flow cytometry. Error bars represent S.E.M. Statistical significance was calculated between untreated and nonmanipulated samples (unt), those processed without histamine (base), and samples treated with histamine with or without JNJ-39758979 using a Student's t test; †$P < 0.05$ relative to untreated samples. Statistical significance between each JNJ-39758979 group and vehicle group (0 JNJ-39758979) was assessed by a one-way analysis of variance with post-hoc Dunnett's test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

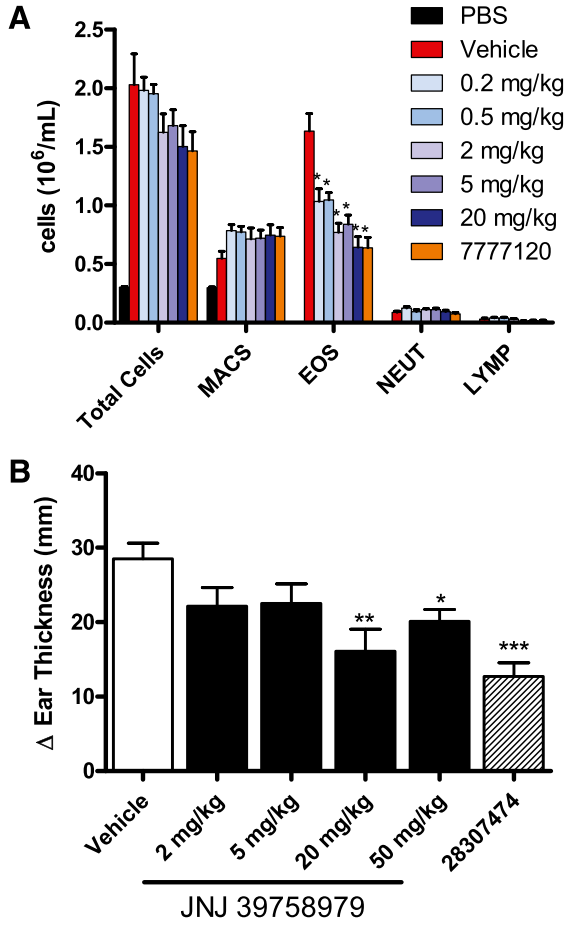

Fig. 3. (A) Female BALB/c mice (n = 10 per group) were sensitized to ovalbumin intraperitoneally on days 0 and 14 before repeat aerosol exposure to ovalbumin on days 21 through 24. Vehicle (20% hydroxypropyl-β-cyclodextran), JNJ-39758979, or JNJ 77777120 (20 mg/kg) was administered orally 20 minutes before each challenge. Some mice (n = 5) were sensitized with ovalbumin, but challenged with phosphate-buffered saline (PBS) only. The total number of cells and a differential cell count were calculated from bronchoalveolar lavage fluid collected 24 hours after the final challenge. (B) Female BALB/c mice (n = 8 mice per group) were sensitized to fluorescein isothiocyanate on days 0 and 1 and then challenged on day 6 by application of fluorescein isothiocyanate to one ear. On day 7 the difference in ear thickness between the challenged and unchallenged ears was measured with calipers. Vehicle (20% hydroxypropyl-β-cyclodextran), JNJ-39758979, or JNJ 28307474 (50 mg/kg) was administered orally 20 minutes prior and 4 hours after fluorescein isothiocyanate application. For both panels statistical significance between each compound-treated group and vehicle was assessed by a one-way analysis of variance with post-hoc Dunnett's test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

pharmacokinetic studies was estimated to be approximately 32–50 nM (7–11 ng/ml) (Savall et al., 2014). These findings suggest that JNJ-39758979 affects inflammatory infiltrates in a model of allergic airway inflammation and may have therapeutic utility in allergic lung disease.

The effects of JNJ-39758979 in a murine model of Th2-dependent contact hypersensitivity have also been investigated. Dermal administration of fluorescein isothiocyanate to sensitized animals resulted in inflammatory edema. Treatment with JNJ-39758979 at doses of 20 and 50 mg/kg twice a day significantly reduced the change in ear edema (Fig. 3B). Data with JNJ 28307474 are shown for comparison and are similar to that previously reported (Cowden et al., 2010b). These data suggest that JNJ-39758979 may be useful in the treatment of Th2-dependent skin diseases, such as atopic dermatitis.

Toxicology. The preclinical pharmacology of JNJ-39758979 suggested that it was a good candidate for clinical development. To support this, preclinical toxicology studies were performed. JNJ-39758979 was evaluated in repeat-dose toxicity studies of up to 6 months duration in rats and 9 months in monkeys. In the GLP 3-month oral toxicity study in rats (0, 10, 50, and 250 mg/kg per day), the no observed adverse effect level (NOAEL) was 50 mg/kg per day (915 and 1290 ng/ml C_{max}, and 5980 and 8490 ng·h/ml AUC_{0-24h} in males and females, respectively). At 250 mg/kg per day (5390 and 5940 ng/ml C_{max} and 66,500 and 79,200 ng·h/ml AUC_{0-24h} in males and females, respectively), findings included decreased body weight; slightly increased levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase; and

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

subacute inflammation and foamy macrophages in the lung. The foamy macrophages correlated ultrastructurally to intralysosomal lamellar bodies consistent with phospholipidosis. There was no evidence of significant hematologic, bone marrow, or lymphoid organ changes. All changes showed partial to full reversal after a 1-month recovery period. In the GLP 6-month toxicity study (doses of 0, 25, 50, 100, and 200 mg/kg per day), clinical pathology findings and the occurrence of foamy macrophages in the lung were similar to those in the 3-month study. The NOAEL in the 6-month study was 100 mg/kg per day for males (2120 ng/ml C_{max}, 17,600 ng·h/ml AUC_{0-24h}) and 200 mg/kg per day for females (4460 ng/ml C_{max}, 49,400 ng·h/ml AUC_{0-24h}).

In GLP oral gavage toxicity studies in monkeys, JNJ-39758979 was administered at doses up to 30 mg/kg per day for 3 months (0, 2, 6, and 30 mg/kg per day) and 9 months (0, 3, 10, and 30 mg/kg per day). No compound-related adverse effects were observed in males at any dose or duration or in females at any dose for 3 months. Females dosed at 30 mg/kg per day for 9 months had decreased body weight. There was no evidence of significant hematologic or bone marrow abnormalities in either study. In particular there were no meaningful differences between bone marrow cytology in the monkeys administered test article at 30 mg/kg per day for 3 months compared with concurrent controls. The NOAEL in male monkeys was the high dose of 30 mg/kg per day for both 3- and 9-month administration (C_{max} 1470 and 1390 ng/ml and AUC_{0-24h} 21,600 and 17,400 ng·h/ml, respectively). The NOAEL in female monkeys was 30 mg/kg per day for 3-month administration (C_{max} 1560 ng/ml and AUC_{0-24h} 21,200 ng·h/ml) and 10 mg/kg per day for 9-month administration (C_{max} 574 ng/ml and AUC_{0-24h} 5920 ng·h/ml).

In both rats and monkeys, there was no evidence of immunotoxicity based on the absence of effects on hematology (Supplemental Tables S6 and S7), bone marrow cytology (Supplemental Table S8), and lymphoid organs (Supplemental Tables S9 and S10). The toxicology data with JNJ-39758979 indicate that there are no significant safety issues related to the inhibition of the H_4R. To further confirm this, studies were carried out to compare C57BL/6 wild-type to H_4R-deficient mice. Mice were compared at approximately 7 weeks of age and 30–43 weeks of age. Five females and five males were used in each group. There were no consistent differences in bone marrow cytology between the wild-type and H_4R-deficient mice at either juvenile or mature phases (Supplemental Tables S11, S12, and S13).

Human Clinical Studies. The preclinical toxicology profile was supportive of further investigation in clinical studies. This, combined with the pharmacology data indicating potential benefit in the treatment of a variety of inflammatory diseases, prompted the initiation of a clinical study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of single and multiple ascending oral doses of JNJ-39758979 in healthy subjects. The study was conducted at a single site in Belgium from September 2008 to June 2009.

The single-dose JNJ-39758979 pharmacokinetic characteristics were evaluated at doses ranging from 10 to 1200 mg with a 5 or 100 mg/ml oral solution under fasting conditions in healthy subjects (Fig. 4 and Supplemental Table S3). JNJ-39758979 was rapidly absorbed into plasma (median T_{max} 0.5–2 hours). Both C_{max} and AUC_{last} in plasma were slightly more than dose proportional between the doses of 30 and 600 mg, but less than dose proportional between the doses of 600 and 1200 mg. The large apparent volume of distribution (V_d/F) indicates extensive tissue distribution of JNJ-39758979. The elimination of JNJ-39758979 appeared to be multiphasic with the mean terminal $t_{1/2}$ ranging from 126 to 157 hours.

In healthy subjects, the multiple-dose pharmacokinetic characteristics of JNJ-39758979 were evaluated at doses ranging from 30 mg daily to 300 mg twice daily for 14 days using an oral solution under fasted conditions (Fig. 5; Supplemental Table S4). JNJ-39758979 was rapidly absorbed into the systemic circulation after multiple oral doses with a median T_{max} of 0.5–2 hours. Both C_{max} and AUC_{0-24h} (days 1 and 14) were increased more than dose proportionally between the doses of 30 and 100 mg daily and 100 and 300 mg daily. Following multiple oral doses of 300 mg twice daily for 14 consecutive days, AUC within 24 hours was approximately 100% greater than that for the 300 mg daily on both days 1 and 14. JNJ-39758979 plasma concentrations appeared to reach steady state by day 14 for all dose levels and regimens evaluated. The accumulation ratio based on AUC_{0-24h} observed on day 1 and day 14 was 3 to 4, corresponding to an effective $t_{1/2}$ ranging from approximately 40 to 60 hours. At the dose of 300 mg per day, steady state C_{max} and AUC_{0-24h} values in healthy female subjects were approximately 20 and 60% greater than those in healthy male subjects, respectively. The mean fluctuation index of JNJ-39758979 was low, ranging from 0.852 to 2.60, suggesting a good maintenance of plasma levels of JNJ-39758979 over time. The elimination of JNJ-39758979 appeared to be multiphasic with the mean terminal $t_{1/2}$ ranging from 134 to 296 hours on day 14 across the doses studied. JNJ-39758979 was detectable in plasma up to 56 days after the last dose on day 14.

Clinical Safety. At single doses ranging from 10 to 1200 mg JNJ-39758979, the only safety and tolerability issues identified were related to gastrointestinal adverse events. No deaths, serious adverse events, discontinuations due to adverse events, or other significant adverse events occurred. Overall, 20 of 35 (57%) subjects in the single-ascending dose study reported one or more adverse events within 7 days of dosing with JNJ-39758979. The most frequently reported adverse events were diarrhea, nausea, headache, abdominal pain, and vomiting

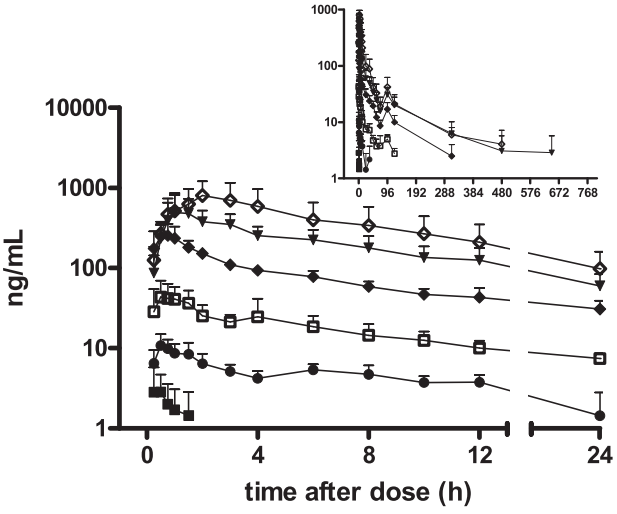

Fig. 4. Plasma concentration of JNJ-39758979 (mean ± S.D.) at various time points after a single oral dose in humans. Doses were 10 mg (■), 30 mg (●), 100 mg (□), 300 mg (♦), 600 mg (▼), and 1200 mg (◊).

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

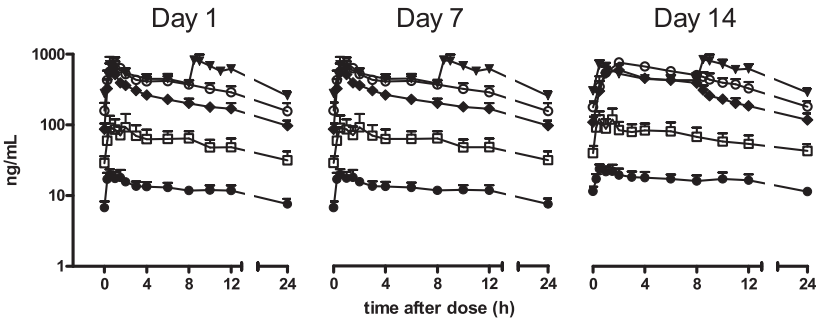

Fig. 5. Plasma concentration of JNJ-39758979 (mean ± S.D.) at various time points after oral doses on days 1, 7, and 14 in the multiple-dose portion of the clinical study. Doses were 30 mg (●), 100 mg (□), 300 mg males (♦), 300 mg females (O), and 300 mg twice daily (▼).

(Table 2). No other consistent, dose-related adverse events were observed. Most events were mild, were self-limited, and occurred in a dose-dependent fashion. No clear or consistent treatment-related changes were observed in vital signs, ECG parameters, mean serum hematology, chemistry, coagulation, urinalysis, or urine microscopy parameters.

Overall, no additional safety issues were identified after multiple oral doses (30–300 mg once daily and 300 mg twice daily) of JNJ-39758979 for up to 14 days. There were no deaths, serious adverse events, discontinuations due to adverse events, or other significant adverse events reported in subjects given JNJ-39758979. Multiple oral doses of JNJ-39758979 ranging from 30 mg to 600 mg (300 mg twice daily) for up to 14 days were safe. The most consistent adverse events of note were gastrointestinal adverse events, chiefly nausea and abnormal feces (Table 3). Headaches also occurred frequently, but at generally similar rates to placebo. Adverse events are summarized up to 7 days after the last dose of JNJ-39758979. No adverse events of insomnia or somnolence were reported, even though JNJ-39758979 does cross the blood-brain barrier. This is in contrast with what is reported with central nervous system–penetrant H_1 or H_3 receptor antagonists. No clear or consistent treatment-related changes were observed in vital signs, ECG parameters, mean serum hematology, chemistry, coagulation, urinalysis, or urine microscopy parameters. Of particular note there was no mean change from baseline absolute neutrophil count following administration of multiple doses of JNJ-39758979 over 14 days at doses up to 300 mg twice daily.

Human Pharmacodynamics. Histamine-induced eosinophil shape change was used as a pharmacodynamic readout in the phase 1 study. At baseline (24 hours prior to the first dose) all treatment groups in both the SAD and MAD portion of the study had a similar percentage change in mean forward scatter upon histamine stimulation, with the exception of the 10-mg single-dose group, which had a mean forward scatter that was statistically higher than that of the placebo group (Fig. 6, A and B). For the SAD cohorts the original planned sampling time points were 3, 6, and 24 hours after the dose. However, a review of the pharmacokinetic data from earlier cohorts indicated that the T_{max} for the compound occurred earlier than 3 hours following dosing. Therefore, starting with the 300-mg cohort, samples were collected at 0.75, 6, and 24 hours after dosing. There was no significant inhibition of the histamine-induced shape change at any time point for the 10-, 30-, or 100-mg cohorts relative to placebo, although the 100-mg cohort showed a trend for inhibition at 6 hours. It should be noted that for these cohorts the effect at the T_{max} for the drug as not assessed. For the 300-, 600-, and 1200-mg dose groups, significant inhibition of the histamine-induced shape change compared with the placebo group occurred at 0.75 and 6 hours. The mean inhibition ranged from 60 to 100%. Inhibition at 24 hours was also noted for these dose groups, but only data from the 600- and 1200-mg cohorts reached statistical significance.

For the MAD portion of the study, samples were collected 1 day prior to dosing on day 1 and on day 14 (0.75, 6, 24, and 48 hours post dose). No inhibition was seen at any time point in the 30-mg group (Fig. 6B). Statistically significant histamine-induced shape change inhibition was observed in the 100, 300, and 300 mg twice-daily cohorts at 0.75 hours (around T_{max}) on the last day of dosing (day 14). The mean inhibition was approximately 75% to 100%. Inhibition as observed at 6 and 24 hours post-dose on day 14 for the 300-mg and 300-mg twice daily groups, but the data for the 300-mg groups did not reach statistical significance at 6 hours. At 24 hours after the day 14 dose, 81.7% inhibition was observed in the 300-mg cohort. In general the data from the 300-mg female cohort were similar to 300-mg male cohort at all time points.

A panel of 98 serum proteins was analyzed in the MAD portion of the study from serum collected predose on days 1, 2, 7, and 14, as well as 4 hours post-dose on day 1. No changes were detected upon treatment with JNJ-39758979, although 22 proteins were below the limit of quantification. A list of the proteins tested is included in Supplemental Table S5.

TABLE 2
Treatment-emergent adverse events with an incidence of >1 in treatment groups after a single oral dose of JNJ-39758979

	Placebo (N = 18)	JNJ-39758979					
		10 mg (N = 6)	30 mg (N = 5)	100 mg (N = 6)	300 mg (N = 6)	600 mg (N = 6)	1200 mg (N = 6)
				n (%)			
Diarrhea	2 (11)	0	0	0	0	4 (67)	5 (83)
Nausea	2 (11)	0	0	0	2 (33)	2 (33)	4 (67)
Vomiting	1 (6)	0	0	0	0	0	4 (67)
Abdominal pain	2 (11)	0	0	0	0	1 (17)	1 (17)
Headache	2 (11)	1 (17)	1 (20)	0	0	2 (33)	0

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

TABLE 3
Treatment-emergent adverse events with an incidence of >1 in treatment groups after 14 days of oral dosing of JNJ-39758979

	Placebo (N = 15)	JNJ-39758979				
		30 mg per Day (N = 6)	100 mg per Day (N = 5)	300 mg per Day (N = 6)	300 mg Twice Daily (N = 6)	300 mg per Day (Female: N = 6)
				n (%)		
Abnormal feces	4 (27)	0	4 (80)	2 (33)	4 (67)	2 (33)
Nausea	2 (13)	0	0	2 (33)	1 (17)	6 (100)
Abdominal pain	2 (14)	1 (17)	0	0	1 (17)	1 (17)
Vomiting	0	0	0	1 (17)	0	1 (17)
Headache	4 (27)	2 (33)	2 (40)	1 (17)	1 (17)	3 (50)
Rash	0	0	0	0	0	2 (33)
Influenza-like illness	0	0	1 (20)	0	0	1 (2)
Malaise	0	0	0	0	1 (17)	1 (2)
Epistaxis	1 (7)	1 (17)	0	0	0	2 (5)
Oropharyngeal pain	0	1 (17)	0	0	0	1 (2)
Oral herpes	0	1 (17)	0	0	0	1 (2)

Discussion

Since its discovery in 2000 it has been suggested that the H_4R is an attractive target for the development of therapies for the treatment of a variety of diseases. Here we describe the preclinical and clinical characterization of one of the first H_4R antagonists to enter the clinic. The clinical data reported here are from a phase 1 study conducted in 2008–2009.

JNJ-39758979 binds to the human H_4R with a high affinity and has good selectivity over other histamine receptors. As for many of the other H_4R ligands, JNJ-39758979 does display differences at the H_4R across the various species. In particular, while the compound has a high affinity for the human, mouse, and monkey receptor, it has low affinity for the rat and dog receptor. The high affinity at the mouse receptor is important for the preclinical in vivo characterization of the compound, but the low affinity in rat and dog impacted the choice of toxicology species. In addition, the compound is an antagonist at the H_4R in all species and does not display any agonist activity. This consistent pharmacology across species is crucial when interpreting preclinical efficacy and toxicology data.

The activity of JNJ-39758979 in vivo was consistent with previous reports of other H_4R antagonists in these models (Dunford et al., 2006; Cowden et al., 2010b). Dose-dependent inhibition of eosinophil influx in a mouse asthma model and ear edema in a mouse dermatitis model were observed. For the asthma model effects were seen starting at doses of 0.2 mg/kg, and the C_{max} for the compound at this dose was estimated to be 32–50 nM (7–11 ng/ml). This is about 10 times higher than the measured K_i for the mouse H_4R (5 nM). Efficacy in the dermatitis model was seen with doses that gave trough concentrations that were around 3-fold higher than C_{max} in the asthma model (170 nM; 38 ng/ml). Experience with a variety of H_4R antagonists in these models suggests that the C_{max} is the most important parameter driving efficacy in the mouse asthma model, whereas trough concentrations were most important in the dermatitis model. This is most likely due to the transient exposure experienced to the aerosolized antigen in the asthma model compared with the hapten in the dermatitis model. These data suggest that targeting a trough concentration of JNJ-39758979 of 10–40 ng/ml would be necessary for efficacy in humans. The efficacy data in these models combined with those previous reported in pruritus and arthritis models (Savall et al., 2014) indicate that the compound could have utility in the treatment of a variety of inflammatory diseases.

JNJ 39738979 was able to inhibit histamine-induced eosinophil shape change in vitro. A statistically significant inhibition was observed starting at 100 nM (~20 ng/ml). This pharmacodynamic marker was also used in the phase 1 clinical study. Inhibition of the shape change was detected, indicating that JNJ-39758979 was able to block the H_4R in humans. In general, inhibition was seen at doses and time points where the plasma concentration was above 20 ng/ml, which is consistent with the in vitro results. This pharmacodynamic assay cannot be performed in mice due to the low numbers of circulating eosinophils and their lack of autofluorescence; however, given the similarity in potency at the human and mouse receptor, one might also expect that plasma concentrations above 20 ng/ml would yield an effect in the mouse. This concentration range is within that predicted from the preclinical efficacy results (10–40 ng/ml) and once again supports this range as being necessary for efficacy in humans. However, this relationship may not hold for every compound because it will depend on the degree of protein binding and tissue distribution. Compounds that are highly protein bound and/or highly distributed to tissues may require higher plasma concentrations to show efficacy in such a pharmacodynamic marker. Nevertheless, for JNJ-39758979, the pharmacokinetic data in combination with the pharmacodynamic data suggest that daily dosing at 100 mg or above would achieve the predicted efficacious steady-state trough plasma levels and would yield target engagement.

JNJ-39758979 was absorbed with a median T_{max} of 0.5–2 hours. The disposition was triphasic with a mean terminal elimination $t_{1/2}$ ranging from 126 to 157 hours across the dose range of 100 to 1200 mg. Reasons for the slow elimination are unknown. Foamy alveolar macrophages consistent with phospholipidosis were observed in rats following large doses of JNJ-39758979. Compounds inducing phospholipidosis are known to accumulate in cells in association with the increased phospholipids (Reasor et al., 2006). However, it is unlikely that the slow elimination is due to phospholipidosis as the long half-life was observed at doses and exposures below those associated with phospholipidosis, but the contribution of lysosomal trapping on slow drug elimination cannot be ruled out.

JNJ-39758979 PK variability was moderate to high, ranging from 31 to 50% for C_{max} and 36 to 53% for area under the curve

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

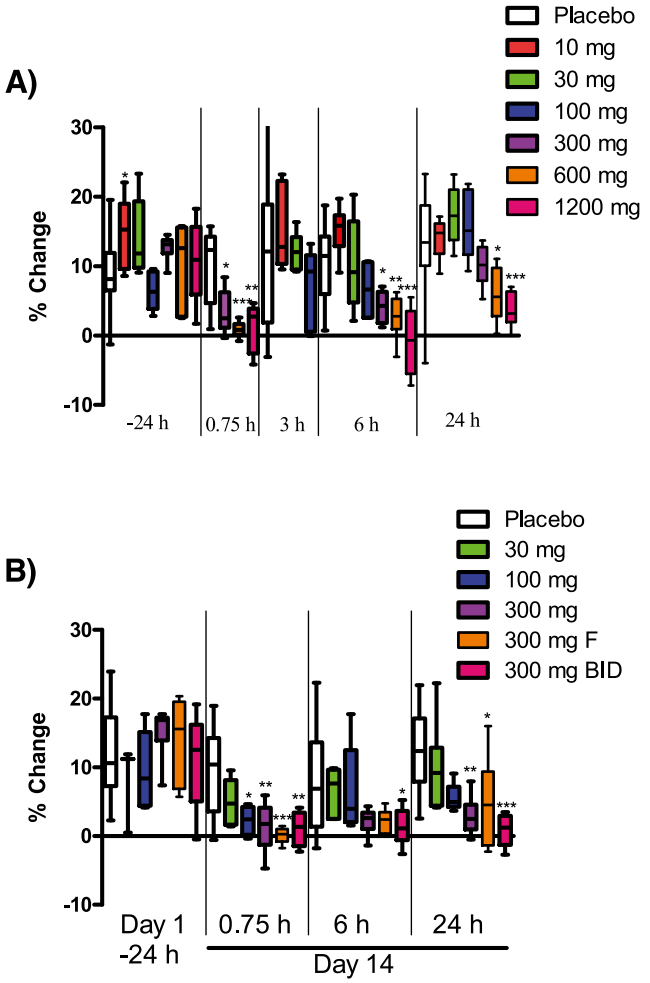

Fig. 6. Histamine-induced eosinophil shape change was assessed during the human clinical study. Blood was stimulated with 0.3μM histamine for 10 minutes and then the forward scattering was assessed by flow cytometry. The percent change in the geometric mean of the forward scatter compared with samples where no histamine was added is plotted as box-and-whisker plots indicating the maximum and minimum responses. (A) Data from the single ascending portion of the clinical study. Data were collected 1 day prior to dosing (−24 hours) and at several time points after dosing. (B) Data from the multiple ascending dose portion of the clinical study. Data were collected 1 day prior to dosing on day 1 (−24 hours) and at several time points after dosing on day 14. The data from the female cohort are indicated by F. All other data are from males. Statistical comparison of treated groups to the placebo group was assessed by a one-way analysis of variance with post-hoc Dunnett's test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

from time 0 to infinity after single-dose administration, while its variability appeared to be lower at steady state, ranging from 7 to 33% for C_{max} and 9 to 24% for AUC_{0-24h} at steady state. Formal statistical analysis on dose proportionality was not done because of limited sample size and high variability. However, both C_{max} and AUC for JNJ-39758979 increased with the increase of dose after single- and multiple-dose administration. The increase of C_{max} and AUC appeared to be greater than dose proportional at the dose range from 10 to 600 mg, but less than dose proportional from 600 to 1200 mg. This observed dose proportionality may be the result of JNJ-39758979 as the substrate of drug transporters; however, more investigation is needed to support this hypothesis.

The safety data from the preclinical toxicity studies and the phase 1 clinical study suggest that H_4R antagonists should have an overall good safety profile in general. Preclinical toxicity studies with JNJ-39758979 in both rat and monkey did not reveal any toxicity associated with either the compound or with antagonism of the H_4R. This was true even with long-term daily administration (6 months rat, 9 months monkey). In general, no adverse findings were observed at any dose outside of decreases in body weight gain. In the clinic, the compound was in general well tolerated up to 1200-mg single dose or 300-mg twice daily dosing for 14 days, and no safety signals were noted. The only tolerability issue observed was a dose-dependent increase in gastrointestinal adverse events that were mainly nausea and vomiting. It is thought that this was a result of a local nonspecific effect in the stomach, and tolerability was greatly improved in later clinical studies with an enteric coating (data not shown). Overall, no on-target related safety issues were identified in this phase 1 study; however, a later clinical study (ClinicalTrials.gov identifier: NCT01497119) did determine that JNJ-39758979 was associated with drug-induced agranulocytosis. The details of this will be disclosed in a separate publication.

In conclusion, JNJ-39758979 is a potent and selective H_4R that exhibited excellent preclinical safety and evidence of a pharmacodynamics effect in humans. Further clinical development of the compound has been terminated because of drug-induced agranulocytosis. Despite the termination of this compound, the data suggest that targeting the H_4R holds promise to deliver safe and effective treatments for a variety of immune-mediated conditions.

Acknowledgments

The authors thank Jeffrey M. Cowden, Jason P. Riley, Patricia McGovern, and Pragnya Desai for technical support.

Authorship Contributions

Participated in research design: Thurmond, Chen, Dunford, Greenspan, Karlsson, La, Ward, Xu.

Conducted experiments: Dunford, Greenspan, Xu.

Performed data analysis: Thurmond, Chen, Dunford, Greenspan, La, Ward, Xu.

Wrote or contributed to the writing of the manuscript: Thurmond, Chen, Dunford, Greenspan, Karlsson, La, Ward, Xu.

References

Ballerini C, Aldinucci A, Luccarini I, Galante A, Manuelli C, Blandina P, Katebe M, Chazot PL, Masini E, and Passani MB (2013) Antagonism of histamine H4 receptors exacerbates clinical and pathological signs of experimental autoimmune encephalomyelitis. *Br J Pharmacol* **170**:67–77.

Cowden JM, Riley JP, Ma JY, Thurmond RL, and Dunford PJ (2010a) Histamine H4 receptor antagonism diminishes existing airway inflammation and dysfunction via modulation of Th2 cytokines. *Respir Res* **11**:86.

Cowden JM, Yu F, Banie H, Farahani M, Ling P, Nguyen S, Riley JP, Zhang M, Zhu J, and Dunford PJ, et al. (2014) The histamine H4 receptor mediates inflammation and Th17 responses in preclinical models of arthritis. *Ann Rheum Dis* **73**:600–608.

Cowden JM, Zhang M, Dunford PJ, and Thurmond RL (2010b) The histamine H_4 receptor mediates inflammation and pruritus in Th2-dependent dermal inflammation. *J Invest Dermatol* **130**:1023–1033.

del Rio R, Noubade R, Saligrama N, Wall EH, Krementsov DN, Poynter ME, Zachary JF, Thurmond RL, and Teuscher C (2012) Histamine H4 receptor optimizes T regulatory cell frequency and facilitates anti-inflammatory responses within the central nervous system. *J Immunol* **188**:541–547.

Dunford PJ, O'Donnell N, Riley JP, Williams KN, Karlsson L, and Thurmond RL (2006) The histamine H_4 receptor mediates allergic airway inflammation by regulating the activation of CD4+ T cells. *J Immunol* **176**:7062–7070.

Dunford PJ, Williams KN, Desai PJ, Karlsson L, McQueen D, and Thurmond RL (2007) Histamine H_4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. *J Allergy Clin Immunol* **119**: 176–183.

Gao Y, Davies SP, Augustin M, Woodward A, Patel UA, Kovelman R, and Harvey KJ (2013) A broad activity screen in support of a chemogenomic map for kinase signalling research and drug discovery. *Biochem J* **451**:313–328.

Ling P, Ngo K, Nguyen S, Thurmond RL, Edwards JP, Karlsson L, and Fung-Leung W-P (2004) Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br J Pharmacol* **142**:161–171.

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019

Matsushita A, Seike M, Okawa H, Kadawaki Y, and Ohtsu H (2012) Advantages of histamine H4 receptor antagonist usage with H1 receptor antagonist for the treatment of murine allergic contact dermatitis. *Exp Dermatol* **21**: 714–715.

Mowbray CE, Bell AS, Clarke NP, Collins M, Jones RM, Lane CAL, Liu WL, Newman SD, Paradowski M, and Schenck EJ, et al. (2011) Challenges of drug discovery in novel target space. The discovery and evaluation of PF-3893787: a novel histamine H4 receptor antagonist. *Bioorg Med Chem Lett* **21**:6596–6602.

Nent E, Frommholz D, Gajda M, Bräuer R, and Illges H (2013) Histamine 4 receptor plays an important role in auto-antibody-induced arthritis. *Int Immunol* **25**: 437–443.

Ohsawa Y and Hirasawa N (2012) The antagonism of histamine H1 and H4 receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice. *Allergy* **67**:1014–1022.

Reasor MJ, Hastings KL, and Ulrich RG (2006) Drug induced phospholipidosis: issues and future directions. *Expert Opin Drug Saf* **5**:567–583.

Rossbach K, Nassenstein C, Gschwandtner M, Schnell D, Sander K, Seifert R, Stark H, Kietzmann M, and Bäumer W (2011) Histamine H1, H3 and H4 receptors are involved in pruritus. *Neuroscience* **190**:89–102.

Salcedo C, Pontes C, and Merlos M (2013) Is the H4 receptor a new drug target for allergies and asthma? *Front. Biosci. Elite Ed.* **E5**:178–187.

Savall BM, Chavez F, Tays K, Dunford PJ, Cowden J, Hack MD, Wolin RL, Thurmond RL, and Edwards JP (2014) Discovery and SAR of 6-alkyl-2,4-diaminopyrimidines as histamine H4 receptor antagonists. *J Med Chem* DOI:10.1021/jm401727m [published ahead of print].

Shin N, Covington M, Bian D, Zhuo J, Bowman K, Li Y, Soloviev M, Qian D-Q, Feldman P, and Leffet L, et al. (2012) INCB38579, a novel and potent histamine H_4 receptor small molecule antagonist with anti-inflammatory pain and anti-pruritic functions. *Eur J Pharmacol* **675**:47–56.

Somma T, Cinci L, Formicola G, Pini A, Thurmond R, Ennis M, Bani D, and Masini E (2013) A selective antagonist of histamine H_4 receptors prevents antigen-induced airway inflammation and bronchoconstriction in guinea pigs: involvement of lipocortin-1. *Br J Pharmacol* **170**:200–213.

Suwa E, Yamaura K, Oda M, Namiki T, and Ueno K (2011) Histamine H(4) receptor antagonist reduces dermal inflammation and pruritus in a hapten-induced experimental model. *Eur J Pharmacol* **667**:383–388.

Thurmond RL, Desai PJ, Dunford PJ, Fung-Leung W-P, Hofstra CL, Jiang W, Nguyen S, Riley JP, Sun S, and Williams KN, et al. (2004) A potent and selective histamine H_4 receptor antagonist with anti-inflammatory properties. *J Pharmacol Exp Ther* **309**:404–413.

Varga C, Horvath K, Berko A, Thurmond RL, Dunford PJ, and Whittle BJR (2005) Inhibitory effects of histamine H_4 receptor antagonists on experimental colitis in the rat. *Eur J Pharmacol* **522**:130–138.

Yamaura K, Oda M, Suwa E, Suzuki M, Sato H, and Ueno K (2009) Expression of histamine H4 receptor in human epidermal tissues and attenuation of experimental pruritus using H4 receptor antagonist. *J Toxicol Sci* **34**:427–431.

Yu F, Wolin RL, Wei J, Desai PJ, McGovern PM, Dunford PJ, Karlsson L, and Thurmond RL (2010) Pharmacological characterization of oxime agonists of the histamine H4 receptor. *J Receptor Ligand Channel Res* **3**:37–49.

Address correspondence to: Robin L. Thurmond, 3210 Merryfield Row, San Diego, CA 92121. E-mail: rthurmon@its.jnj.com

Downloaded from jpet.aspetjournals.org at ASPET Journals on March 25, 2019