Pharmacology and Clinical Activity of Toreforant, a Histamine H4 Receptor Antagonist

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Abstract

Toreforant is a potent and selective histamine H4 receptor (H4R) antagonist with a Ki at the human receptor of 8.4 ± 2.2 nM and excellent selectivity over other receptors including the other histamine receptors. The compound acts as an antagonist in all species tested and inhibits histamine-induced eosinophil shape change in vitro. Toreforant was anti-inflammatory in mouse models of asthma and arthritis. However, it was not able to inhibit histamine-induced scratching in mice or block neuropathic pain in rats. The lack of effect in these models may be related to low exposure levels in the central nervous system. Preclinical toxicity studies of up to 6 months in rats and 9 months in monkeys indicated an excellent safety profile with the exception of QT prolongation seen in vivo due to inhibition of the human ether-a-go-go-related gene (hERG) channel. Toreforant was studied in phase 1 human clinical studies to assess safety, pharmacokinetics and pharmacodynamics. The compound was well-tolerated at all doses tested and no safety issues were noted in the phase 1 studies with the exception of QT prolongation observed at the highest dose. Toreforant exhibited good pharmacokinetics upon oral dosing with a plasma half-life consistent with once a day dosing. In addition, dose-dependent inhibition of histamine-induced eosinophil shape change was detected suggesting that the H4R was inhibited in vivo.

Abbreviations

H4R: Histamine H4 Receptor; MAD: Multiple Ascending Dose; SAD: Single Ascending Dose; GLP: Good Laboratory Practice; NOAEL: No Observed Adverse Effect Level; Cmax: Maximum Concentration; Cmin: Minimum Concentration; AUC0-24h: Area Under the Curve from Time 0 to 24h; Tmax: Time at Which Maximum Plasma Concentration is Observed; AUClast: Area Under the Curve from Time 0 to the Last Measured Time Point; V/F: Volume Of Distribution; CL: Terminal Half-Life; CNS: Central Nervous System; K: Inhibition Constant; IC50: Concentration Producing 50% of Maximum Inhibitory Effect; pA2: Negative Logarithm of the Concentration Needed To Shift The Histamine Dose Response 2-Fold; hERG: Human Ether-À-Go-Go-Related Gene; ECG: Electrocardiogram; BAL: Broncho Alveolar Lavage; AE: Adverse Event; QTcF: QT Interval Corrected for Heart Rate Using the Fredericia Formula; SEM: Standard Error of the Mean

Introduction

The histamine H4 receptor (H4R) is a class A G-protein coupled receptor that was discovered via genomic means and was found to bind to histamine, thus making it the fourth receptor for this ligand [1]. The H4R has been implicated in driving inflammatory responses under a number of different conditions [1]. The receptor has activity in several cell types involved in inflammation including mast cells, eosinophils, dendritic cells and T cells. In addition, there are reports of effects of H4R antagonists in pruritic and pain models [2,3]. Thus, there are many potential therapeutic uses for H4R antagonists.

To date only a handful of H4R antagonists have reached the clinic. Clinical studies have been reported in pruritus, atopic dermatitis, rheumatoid arthritis, asthma, allergic rhinitis and psoriasis. The H4R antagonist JNJ 39758979 has exhibited efficacy in atopic dermatitis and in blocking histamine-induced itch [4-6]. Clinical data have been reported with toreforant, a novel H4R antagonist, in rheumatoid arthritis where an initial small study suggested evidence of efficacy although it was terminated early, but a subsequent study did not show benefit over placebo [7]. The current work describes the detailed pharmacology of toreforant (pronunciation - tor ef’ oh rant), also known as TOREFORANT, and reports the phase 1 clinical safety, pharmacokinetic and pharmacodynamics data for this selective H4R antagonist.
Materials and Methods

Materials

Toreforant (Figure 1) was synthesized as described [8]. For the animal pharmacology models, mice were housed in community cages on a 12 h light/dark cycle. Food and water were available ad libitum. All procedures were performed according to the internationally accepted guidelines for the care and use of laboratory animals in research and were approved by the local IACUC.

In vitro Pharmacology

Binding and functional assays for the various histamine receptors, the muscarinic receptors and the panel of 50 different biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and neurotransmitter transporter binding assays were carried out as previously described [6,9,10]. Histamine-induced eosinophil shape change was conducted as previously described [6,10,11].

Mouse asthma and arthritis models

The ovalbumin mouse asthma model and the collagen-induced arthritis model were conducted as previously described [12,13]. Toreforant was dosed orally in 20% hydroxypropyl-β-cyclodextrin. For the asthma model the compound was administered 20 min prior to the daily allergen challenge. In the collagen-induced arthritis model toreforant was given orally twice a day starting with the first signs of disease onset around Day 30 and continued for 14 days.

Pruritus models

The histamine-induced pruritus model in mice was conducted as previously described [2]. CD-1 mice (5 per group) were dosed with vehicle (20% hydroxypropyl-β-cyclodextrin), toreforant or JNJ 28307474, as a positive control, at 50 mg/kg orally, 60 mins prior to the intra-dermal injection of histamine. Bouts of scratching were recorded over a 20 minute period. In a subsequent experiment mice were orally dosed with toreforant (100 mg/kg) at 24, 8, 4 and 1 h prior to intra-dermal injection of histamine and similarly monitored. In addition compound 48/80-induced scratching was assessed in female Mdr-1a/1b (P-glycoprotein) knockout mice and wild-type (FVB/N) mice. The area at the back of the neck of mice was shaved 24 hours prior to an intra-dermal injection of 20 µl of 100 µg compound 48/80. Both knockout and wild-type mice (five mice per group) were dosed with vehicle (20% hydroxypropyl-β-cyclodextrin), toreforant (50 mg/kg) or JNJ 28307474 (50 mg/kg), orally, 60 mins prior to the intra-dermal injection. Bouts of scratching were recorded over a 20 minute period. Terminal (t=80 min) plasma and brain samples were analyzed for drug concentration.

Spinal Nerve Ligation Model (SNL; Chung Model) of Neuropathic Pain

Male Sprague - Dawley rats (Harlan Laboratorie, Indianapolis, IN) were deeply anesthetized with isoflurane/oxygen inhalational anesthesia. The left lumbar spinal nerves L5 and L6 were tightly ligated (6-0 silk suture) distal to the dorsal root ganglion and prior to their entrance into the sciatic nerve, as described by Kim and Chung [14]. The incisions were closed and the rats were allowed to recover for 2 weeks under conditions described above. Rats were included in the study only if they did not exhibit motor dysfunction (e.g., paw dragging) and only if they had a post-injury threshold less than 3.0 g.

Mild thermal injury

Male Sprague-Dawley rats (Harlan) were deeply anesthetized with isoflurane/oxygen inhalational anesthesia and the left hind paw was placed on a Hot-Plate Analgesia Meter (Columbus Instruments, Columbus, OH) set at 56°C for 25 s with an 80 gram weight on top of the paw.

Carrageenan model of inflammatory pain

For carrageenan inflammation of the paw, carrageenan was purchased from Wako Pure Chemical Industries, L.L.C. (Japan) and was dissolved in 0.9% saline at a ratio of 1g/100 mL (1% solution w/v). The solution was chilled to 4°C for 1 hour to reach a stable viscous state. Prior to subcutaneous injection into the left hind paw in a volume of 100 µL, carrageenan solution was brought to room temperature.

Sprague-Dawley rats (Harlan) were deeply anesthetized with isoflurane/oxygen inhalational anesthesia and injected subcutaneously with 100 µL of 1% carrageenan into the plantar aspect of the left hind paw.

Spared Nerve Injury Model (SNI) of Neuropathic Pain

Male C57/bl6 mice (Charles River Laboratories, Wilmington, MA) were used for the spared nerve injury experiments. Mice were deeply anesthetized with isoflurane/oxygen inhalational anesthesia. The left common peroneal and left sural branches of the sciatic nerve were transected, sparing the tibial branch of the sciatic nerve as previously described [15]. The incisions were closed and the mice were allowed to recover for 2 weeks under conditions described above. Mice were included in the study only if they did not exhibit motor dysfunction (e.g., paw dragging) and only if they had a post-injury threshold less than 0.9 grams.

Tactile threshold testing

In rats mechanical (tactile) allodynia was assessed by determining the median threshold at which the affected paw was withdrawn from 8 graded stimuli (von Frey filaments: 0.4, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0, and 15.0 g; Stoeiling Co, Wood Dale, IL) applied perpendicularly with sufficient force to bend slightly and held for 5-7 seconds against the plantar hindpaw through wire-mesh observation cages. Paw flinching during or immediately following the removal of the stimulus was considered a positive response. A 50% paw withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength and analyzing withdrawal data using an adaptation of the Dixon up-down method [16,17]. Rats were included in the study only if their post-injury paw withdrawal threshold was less than 3.0 g.

Tactile allodynia was assessed in mice similarly as in rats; however, 7 graded stimuli (von Frey filaments: 0.07, 0.16, 0.4, 0.6, 1.0, 2.0 and 4.0 g; Stoeiling) were applied for only about 2-3 seconds. Mice

Figure 1: The structure of the H4R antagonists toreforant, JNJ 39758979, JNJ 7777120 and JNJ 28307474.

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were included in the study only if they had a post-injury threshold less than 0.9 grams. For all studies the tactile thresholds (log value) were normalized for each animal to a percent of a maximum possible effect (%MPE):

\[
\text{\%MPE} = \left( \frac{\text{Threshold(\text{t})} - \text{Threshold(\text{base})}}{\text{Threshold(\text{pre})} - \text{Threshold(\text{base})}} \right) \times 100\%
\]

Where:

\( \text{t} \) = post-treatment time

\( \text{Threshold (t)} = \log \) of the calculated 50% threshold at a time point following compound or vehicle administration

\( \text{Threshold (base)} = \log \) of the calculated 50% threshold taken at baseline; i.e. post-injury and prior to administration of compound or vehicle

\( \text{Threshold (pre)} = \log \) of the calculated 50% threshold taken before model induction, i.e. “preinjury”

A 100% value indicates a complete “anti-allodynia” or return from allodynia to preinjury threshold values. It is possible for some drugs, such as morphine, to produce increases in threshold values above preinjury values or those in otherwise naïve animals. Data were expressed as mean ± standard error of the mean (SEM) and group differences were compared over time using two-way ANOVA followed by Bonferroni post hoc tests with a significance level of p < 0.05 using Prism 5.01 (GraphPad; LaJolla, CA).

**Preclinical cardiovascular assessments**

HEK293 cells stably expressing the human ether-a-go-go-related gene (hERG) were plated at low density onto glass cover slips 24-72 h prior to recording. On the day of the experiment, glass cover slips containing HEK-hERG were placed in a bath on the stage of an inverted microscope and perfused (approximately 1 ml/min) with extracellular solution of the following composition: 137 mM NaCl, 1.8 mM CaCl₂, 4 mM KCl, 1 mM MgCl₂, 5 mM glucose, and 10 mM HEPES, pH 7.4, with NaOH, 306 mOsm. Pipettes were filled with an intracellular solution of the following composition: 120 mM KCl, 2 mM MgCl₂, 5 mM EGTA, 10 mM HEPES, 4 mM Mg ATP, pH 7.3 to 7.4 with KOH, ~290 mOsm, and had a resistance of 2 to 4 MΩ. All recordings were made at room temperature (22-24°C) using a Multi clamp 700 A amplifier and PCLAMP9 software (Axon Instruments). Cells were voltage clamped at a holding potential 80 mV and hERG current was activated by a depolarizing step first to 50 mV for 500 msec, a step to +60 mV for 2 sec to activate the channels, and then finally back to 50 mV for 6 sec to remove the inactivation and observe the deactivating tail current. Voltage protocols were repeated every 15s. The first step at ~50 mV was used as baseline for measuring the tail current peak amplitude. Toreforant was diluted from 10 mM dimethylsulfoxide stock solutions into extracellular solution and applied to cells using a SF- 77B Fast-Step Perfusion device (Warner Instruments). Maximum final dimethylsulfoxide concentration was 0.1%. Two to five cells per concentration of toreforant were used.

Electrophysiological experiments were performed on isolated rabbit Purkinje fibers using conventional microelectrode techniques to record transmembrane action potentials using an approach previously described [18]. A cardiovascular assessment in anesthetized dogs was carried out to determine the electrophysiological and hemodynamic effects of JNJ-38518168 following 5-min intravenous cumulative infusions at 0.08, 0.16, 0.32, 0.63, 1.25 and 2.5 mg/kg in anesthetized dogs (n = 4). For each dog a baseline was established for at least 20 min. The test article starting with vehicle only was administered as a 5 min infusion and data was collected at 5 and 30 min afterwards before the next dose. Toreforant was also administered orally via gavage to male beagle dogs (4/4 dose) at doses of 3, 10 or 100 mg/kg. Electrocardiograms were obtained from cutaneously placed biopotential leads and recorded continuously for approximately 30 minutes before dosing till approximately 6 hours after start of dosing.

**Toxicology studies**

Toreforant 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 at 0, 10, 100 mg/kg/day (N=10/sex/group) for 1 month and 3 months. Two 6 month studies (N=20/sex/group) were conducted at 0, 10 and 100 mg/kg/day for the first study and 25, 50 and 75 mg/kg/day for the second study. Studies in monkeys were conducted at 0, 10, 30, 100 mg/kg/day for 3 months (N=4/sex/group) and 9 months (N=4/sex/group). Toreforant 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. Toreforant was assessed for potential effects on fertility and reproductive capacities, including conception and implantation, in GLP studies in male and female rats as well as being assessed for potential effects on embryofetal development in studies in pregnant rats and rabbits. The potential for Toreforant to cause adverse neurobehavioral effects was assessed in a modified Irwin’s test in male Sprague-Dawley rats. Male rats (5/group) received single oral doses of 0 (vehicle), 3, 30, or 300 mg/kg. A functional observation battery or modified Irwin screen parameters, plus locomotor activity were monitored before dosing and at 2, 4, 6, 8 and 24 hours after dosing, and on Day 7 for any possible delayed findings. Lastly, JNJ-38518168 was evaluated for carcinogenicity in rats dosed at 0, 5, 15, or 50 mg/kg/day (65/group) for 2 years. All of these studies were conducted in compliance with good laboratory practice (GLP) regulations.

**Clinical studies**

Two single-center, double-blind, randomized, placebo-controlled studies were conducted in Belgium between January, 2008 and January, 2009. Both studies were conducted according to Declaration of Helsinki principles and International Committee on Harmonization Good Clinical Practice guidelines. Governing ethical bodies at each study site approved the protocol(s), and all patients provided written informed consent before the conduct of study-specific procedures. Toreforant was supplied as a capsule formulation. In the single ascending dose (SAD) study, eligible male subjects in each treatment group were randomized to receive either a single oral dose of toreforant or placebo, after an overnight fast. The doses were 5, 15, 50 and 150 mg with 7 subjects receiving study drug and 3 placebo in each group with the exception of the 150 mg group where 5 subjects received study drug and 3 subjects received placebo. Two additional cohorts were enrolled at 150 mg with 7 subjects receiving study drug and 3 placebo in each cohort (10 subjects per dose group). The doses of toreforant were escalated in a stepwise fashion if the safety, tolerability and plasma pharmacokinetic profile (up to 24 hours) were deemed acceptable. The main criteria for inclusion in the study were healthy male subjects between 18 and 55 years of age, inclusive; body mass index within 18 to 29 kg/m², with a minimum body weight of 50 kg; supine blood pressure (after resting for 5 minutes) between...
the range of 90 to 139 mm Hg systolic and 50 to 89 mm Hg diastolic, inclusive. In the multiple ascending dose (MAD) study, male subjects received oral doses of placebo or 15, 50,100, and 300 mg of toreforant and female subjects received placebo or 100 mg of toreforant for 14 consecutive days. The doses were escalated in a stepwise fashion if the safety, tolerability and plasma pharmacokinetic profile were deemed acceptable. Each cohort consisted of 10 subjects (7 on study drug and 3 on placebo). The main criteria for inclusion in the study were similar to the SAD study except healthy postmenopausal or non-childbearing (i.e. surgically sterile) female subjects were enrolled in addition to male subjects. For both studies, 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, neurological examination, 12-lead electrocardiogram (ECG), continuous ECG monitoring (telemetry), vital signs (blood pressure, heart rate, respiratory rate, and temperature), clinical laboratory tests including 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 (females), 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 both clinical studies, venous blood samples were taken for the measurement of toreforant plasma concentrations. A validated, specific, and sensitive liquid chromatography-mass spectrometry/mass spectrometry method was used for analysis of plasma samples to determine concentrations of toreforant. Pharmacokinetic parameters were determined from plasma data of toreforant after single oral administration or after multiple oral administrations. The maximum concentration (Cmax) and time of maximum concentration (Tmax), area under the curve from time 0 to 24 h (AUC0-24), and the area under the curve from time 0 to the last measured time point (AUClast) were reported.

Pharmacodynamic assay

Venous blood samples were collected into potassium EDTA tubes at the time points specified. The histamine induced eosinophil shape change was measured as previously described [6]. Statistical analysis was carried out using a one-way ANOVA with post-hoc Dunnett’s test.

Results

**In vitro Pharmacology**

The pharmacological activity of toreferant at the histamine H1, H2, H3, and H4 receptors was investigated in various species (Table 1). These findings indicate that toreforant has high affinity for the human, rat, monkey, and guinea pig H4R, but weaker affinity for the mouse and dog H4R. The compound is a selective H4R antagonist in all species tested. Toreforant did exhibit weak affinity for the human or guinea pig histamine H3 receptor up to concentrations of 10,000 nM, but it does have weak affinity for the mouse histamine H1 receptor with inhibition constant (Ki) value of 3993 nM.

Table 1: Ki, EC50, and pA2 values for toreforant at histamine receptors from various species.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Species</th>
<th>Ki (nM)</th>
<th>EC50 (µM)</th>
<th>pA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>H4</td>
<td>Human</td>
<td>8.4 ± 2.2</td>
<td>&gt;10</td>
<td>8.15 ± 0.11</td>
</tr>
<tr>
<td>H4</td>
<td>Mouse</td>
<td>307 ± 42</td>
<td>&gt;10</td>
<td>6.39 ± 0.02</td>
</tr>
<tr>
<td>H4</td>
<td>Rat</td>
<td>9.3 ± 3.2</td>
<td>&gt;10</td>
<td>6.74 ± 0.27</td>
</tr>
<tr>
<td>H4</td>
<td>Dog</td>
<td>680 ± 138</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H4</td>
<td>Guinea Pig</td>
<td>5.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H4</td>
<td>Monkey</td>
<td>10.6</td>
<td>ND</td>
<td>8.05</td>
</tr>
<tr>
<td>H3</td>
<td>Human</td>
<td>215</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H3</td>
<td>Mouse</td>
<td>1404</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H3</td>
<td>Rat</td>
<td>874</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>H2</td>
<td>Human</td>
<td>&gt;1000</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H1</td>
<td>Human</td>
<td>&gt;10,000</td>
<td>&gt;100,000</td>
<td>&lt;4</td>
</tr>
<tr>
<td>H1</td>
<td>Mouse</td>
<td>3993</td>
<td>&gt;100,000</td>
<td>&lt;4.5</td>
</tr>
<tr>
<td>H1</td>
<td>Guinea Pig</td>
<td>&gt;100,000</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Ki = inhibition constant, EC50 = concentration producing 50% of the maximum possible effect for inhibition of cyclic adenosine monophosphate (cAMP) production in transfected cells, pA2 = negative logarithm of the concentration needed to shift the histamine dose response 2-fold in inhibiting cAMP production in transfected cells, H4 = histamine 4, H3 = histamine 3, H2 = histamine 2; H1 = histamine 1, ND = not determined.

aData given as ± SEM if the assay was run at least 2 times.

Figure 2: The effects of toreforant on histamine-induced eosinophil shape change. (A) Human polymorphonuclear leukocytes were stimulated with various concentrations of histamine in addition to 0µM (□), 0.03 µM (♦), 0.1 µM (○), 0.3 µM (▼), 1 µM (▲), 3 µM (▲) or 10 µM (●) toreforant. After 5 min the percent increase in forward scatter (FSC) was measured by flow cytometry and the mean is plotted. (B) Human blood was incubated with various concentrations of toreforant prior to stimulation with either 0.1 µM (▼) or 0.3 µM (●) histamine. After 5 min the percent increase in forward scatter (FSC) was measured by flow cytometry and the mean is plotted.
Inhibited the histamine-induced shape change of human eosinophils [11, 19]. In human polymorphonuclear leukocytes toreforant chemotaxis of eosinophils, and this can be represented by a change in a good separation between inhibition of the H4R and other receptors.

Toreforant is not a ligand for the human histamine H2 receptor up to concentrations of 1,000 nM.

To determine the role of H4R in autoimmune arthritis, toreforant was studied in a mouse collagen-induced arthritis model. Toreforant was given orally twice a day starting with the first signs of disease onset around Day 30 and continued for 14 days. The animals treated with 100 mg/kg toreforant had reduced disease severity scores (Figure 3b). The reduction is scores was similar to the control H4R antagonist, JNJ 28307474, and with previously published data [6, 13]. The joints were also analyzed by histology and toreforant at 100 mg/kg led to decreases in inflammation, pannus, cartilage damage, and bone resorption (data not shown). Modeling of the mouse exposure at this dose based on separate mouse pharmacokinetic studies was 28307474. The IC50 values are quite high given the affinity for toreforant as Cmin was given orally twice a day.

One of the known activities of the H4R is the ability to induce chemotaxis of eosinophils, and this can be represented by a change in cell shape [11, 19]. In human polymorphonuclear leukocytes toreforant inhibited the histamine-induced shape change of human eosinophils and produced a rightward shift in the histamine dose response curves indicating that it is acting as an antagonist of the human H4R in these primary cells (Figure 2a). This is not an equilibrium measurement and therefore the calculation of a pA2 is complicated. The pA2 can be estimated using the shift seen the lowest concentration of antagonist. This yields a pA2 of around 7.5 consistent with the results in the transfected system (Table 1). This assay can also be performed in whole blood and, as for the purified cells, toreforant is able to inhibit the actions of histamine. The IC50 values were 296 nM and 780 nM when 100 nM and 300 nM histamine were used, respectively (Figure 2b). These IC50 values are quite high given the affinity for toreforant for the H4R receptor. However, this can be reconciled by the fact that toreforant is highly protein bound in all species (Table S3). When this is taken into account, a Ki value can be estimated by the difference in the IC50 values at the two histamine concentrations. This yields an estimate of 11 nM that is very close to the value measured in the binding assay of 8 nM (Table 1).

**In vivo Pharmacology**

Previous H4R antagonists have shown activity in an ovalbumin-induced lung inflammation in mice and therefore, the effect or toreforant was explored in this model [6, 12]. Mice (n = 10 per group) were sensitized by intraperitoneal injection of ovalbumin/alum on Day 0 and Day 14. On Day 21 through 24 mice were pre-treated 30 min with vehicle or toreforant prior to a once daily 20 min challenge with phosphate-buffered saline or ovalbumin. The compound was administered orally at 2, 5, 20, 50, and 100 mg/kg. Twenty-four-hour after the last challenge, mice were euthanized and the total number of cells as well as a differential cell count in the bronchoalveolar lavage (BAL) fluid was determined. After challenge with ovalbumin there was a dramatic increase in the total number of cells in the BAL fluid that was mainly due to an increase in the number of eosinophils. The total BAL cells were reduced at doses of 5 mg/kg toreforant and above (Figure 3a, Table S4). The number of eosinophils, neutrophils and lymphocytes were reduced at doses of 5 mg/kg and above. These results are consistent with published data with other H4R antagonists and with experiments in H4R deficient mice [6, 12]. At 5 mg/kg the Cmax of the compound based on separate pharmacokinetic studies was 300 nM (117 ng/mL).

To determine the role of H4R in autoimmune arthritis, toreforant was studied in a mouse collagen-induced arthritis model. Toreforant was given orally twice a day starting with the first signs of disease onset around Day 30 and continued for 14 days. The animals treated with 100 mg/kg toreforant had reduced disease severity scores (Figure 3b). The reduction is scores was similar to the control H4R antagonist, JNJ 28307474, and with previously published data [6, 13]. The joints were also analyzed by histology and toreforant at 100 mg/kg led to decreases in inflammation, pannus, cartilage damage, and bone resorption (data not shown). Modeling of the mouse exposure at this dose based on separate mouse single dose pharmacokinetic studies, yielded an estimated Cmax of 50 µM and the minimum plasma concentration (Cmin) value 24 h after the first dose was 550 nM. These concentrations are quite high, but consistent with the low affinity for the compound at the mouse H4R (Table 1). Experience with other H4R antagonists in this model suggests that Cmin is the most important parameter.

A model of histamine-induced scratching in CD-1 mice (n = 5 per group) was used to judge the anti-pruritic effects of toreforant. The compound was given orally 60 min before an intra-dermal injection of histamine. Bouts of scratching were counted by visual observation.
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Figure 4: A) Assessment of histamine-induced scratching in CD-1 mice (n=5 per group) by vehicle, toreforant or JNJ 28307474. B) Assessment of compound 48/80-induced scratching in wild-type (WT) and PgP-deficient (KO) mice by vehicle (veh), toreforant (Tor) or JNJ 28307474 (7474). Five mice were used per group. For both panels compounds were given p.o. at 50 mg/kg 60 min before pruritogen challenge and the mean number and SEM of bouts of scratching over a 20 min period after histamine injection is plotted. *, indicates p-values <0.001 and †, < 0.05 from a 1-way ANOVA test.

over a 20 min time span. Unlike other H4R antagonists, toreforant was not efficacious in reducing histamine-mediated pruritus (Figure 4a). Even higher doses up to 100 mg/kg given four times over 24 h did not show any anti-pruritic activity (data not shown). In this experiment the positive control, JNJ 28307474, showed maximum efficacy at 20 mg/kg.

Figure 4: A) Assessment of histamine-induced scratching in CD-1 mice (n=5 per group) by vehicle, toreforant or JNJ 28307474. B) Assessment of compound 48/80-induced scratching in wild-type (WT) and PgP-deficient (KO) mice by vehicle (veh), toreforant (Tor) or JNJ 28307474 (7474). Five mice were used per group. For both panels compounds were given p.o. at 50 mg/kg 60 min before pruritogen challenge and the mean number and SEM of bouts of scratching over a 20 min period after histamine injection is plotted. *, indicates p-values <0.001 and †, < 0.05 from a 1-way ANOVA test.

One difference between toreforant and JNJ 28307474 is that toreforant does not cross the blood-brain barrier. After oral administration to rats, toreforant-derived radioactivity was widely distributed into tissues; however, it was not quantifiable in cerebellum, cerebrum, medulla, and spinal cord in either Long Evans or Sprague Dawley rats, suggesting that drug-derived radioactivity does not cross the blood-brain barrier. This is most likely due to toreforant being a strong substrate for P-glycoprotein. The permeability of toreforant across Caco-2 cell monolayers showed a concentration dependent increase at a concentration range of 3 to 100 mM, suggesting that efflux transporters decrease permeability of toreforant at low concentrations (data not shown). Similarly, inhibition of P-glycoprotein in Caco-2 and MDRI transfected MDCK cell monolayers with GF120918 markedly increased apical to basolateral permeability of toreforant at 1 and 5 mM, respectively (data not shown). To test whether central nervous system (CNS) exposure was necessary for anti-pruritic activity, the CNS exposure of toreforant was increased with P-glycoprotein-deficient mice (0.7 ± 0.1 µM in brain of wild-type mice compared to 12 ± 1 µM in P-glycoprotein-deficient mice, plasma concentrations were similar). In wild-type mice toreforant was unable to block 48/80 induced scratching whereas [JN] 28307474 was effective (Figure 4b). However, in P-glycoprotein-deficient mice significant anti-pruritic activity was observed with toreforant, although it was not complete. These results indicate that toreforant was not able to block scratching in mouse models of pruritus and that this lack of effect was probably due to poor CNS penetration with the compound.

CNS activity of the H4R may also plan a role in the analgesic effect reported for H4R antagonists in several models [3]. Neuropathic pain models in rats were conducted with toreforant and an H4R antagonist that does cross the blood-brain barrier, JNJ 39758979. In a rat spinal nerve ligation model JNJ 39758979 was able to significantly attenuate the mechanical allodynia induced in the model, however toreforant had no activity (Figure 5a and b). The same is true for a rat mild-thermal injury model (Figure 5c and d). This is in spite of the fact that toreforant is more potent at the rat H4R compared to JNJ 39758979 (Table 1) [6]. These data are consistent with the pruritus data and may suggest that CNS penetration of H4R antagonists is necessary for both analgesic effects and anti-pruritic activity. The compounds did not exhibit any activity in a mouse spared nerve injury model (data not shown) or a rat carrageenan inflammatory pain model (Figure 5e and f). The lack of activity of JNJ 39758979 in the carrageenan model is surprising given that JNJ 7777120 has been reported to be active in this model, however the model reported here assessed mechanical allodynia whereas the previously reported data assessed thermal hyperalgesia [3].

Preclinical safety

Whole-cell voltage clamp techniques were used in determining the potential effects of toreforant on hERG channel current flow in hERG-transfected HEK293 cells. These studies indicated that toreforant inhibited the hERG mediated potassium (K+J current with an IC50 of 1.5 µM. The action potential (ADP 90) in rabbit Purkinje fibers was prolonged by toreforant in vitro at concentrations of 10 µM and the QT interval corrected for heart rate using a Bazett correction was increased in dogs when given at 2.5 mg/kg intravenously (18% increase; plasma levels at 5 min were 7.7 M) or 100 mg/kg orally (18% in one of four dogs; plasma level was 7 M). These results suggested that toreforant has the potential to increase the QT interval in humans.

The potential for toreforant to cause adverse neurobehavioral effects was assessed in a modified Irwin test in rats. There were no effects on behavioral, neurologic, or autonomic parameters at doses up to 300 mg/kg. Acute and repeat-dose oral toxicity was evaluated in rats, dogs, and monkeys. The maximum nonlethal dose in rats, dogs, and monkeys was 1000, 600, and 300 mg/kg, respectively. In repeat-dose studies in rats, the compound was well tolerated at doses up to 50 mg/kg/day for 6 months. Notable effects at doses above the no observed adverse effect level (NOAEL) of 50 mg/kg/day for 6 months, included decreased body weight/body weight gain, changes in organ weights (adrenal glands, heart, kidney, liver, lung, and ovaries), renal tubular injury (epithelial vacuolation and dilatation), foamy macrophages in multiple organs (ileum, liver, lung, lymph nodes (mandibular, mesenteric, popliteal), spleen, ovaries, and uterus).
minimal inflammation in the lungs, and adrenocortical hypertrophy. After a 3-month recovery period, changes were partially or completely reversed. In a 9 month study in monkeys, the NOAEL was the highest dose tested (100 mg/kg/day); findings included decreased body weight/body weight gain (males), increased kidney and liver weights without histopathology correlate (females), minimal to slight foamy macrophage accumulation in the spleen, and some changes in clinical pathology parameters.

Fertility studies were conducted in male and female rats administered oral doses of up to 100 mg/kg/day. No effects on fertility or early embryonic development were noted in either study, indicating that the NOAEL for maternal toxicity and for fertility and reproductive capacities in both male and female rats was considered to be >100 mg/kg/day. Results from embryo-fetal toxicity studies in rats and rabbits revealed no major abnormalities. Minor abnormalities and variations were observed, but were attributed to maternal toxicity (denoted by decreases in body weight gain, food consumption, corrected mean maternal weight gain, and/or mortality and clinical observations).

A rat 2 year carcinogenicity study evaluated doses of 0, 5, 15, and 50 mg/kg/day. There were no compound-related effects on mortality rates, and histomorphologic changes were consistent with those observed in the rat 1, 3, and 6-month studies. There were no neoplastic changes that were considered attributable to JNJ-38518168.

Phase 1 Clinical studies

Toreforant was studied in both a single ascending dose study in healthy male subjects and a multiple ascending dose study in healthy male and female subjects. In the phase 1 single-dose escalation study in healthy men, 58 subjects were enrolled and completed the study. The dose levels were 5, 15, and 50 mg (7 subjects per dose group), and 150 mg (three cohorts with a total of 19 subjects). The phase 1 multiple-dose escalation study investigated once-daily doses of 15, 50, 100 and 300 mg, for 14 consecutive days in healthy men, and once-daily doses of 100 mg in healthy women. No deaths, serious adverse events, or other significant adverse events (AE) were observed in either study with healthy subjects.

No significant safety concerns were revealed in single ascending dose study. Toreforant was well tolerated at all dose levels from 5 to 150 mg. No deaths, serious adverse events, or discontinuations due to AEs were reported during the study. Overall, 12 (20.7%) of the 58 subjects who received a dose of study agent reported at least 1 AE. The
percentage of subjects who reported AEs was slightly higher overall in the toreforant-treated group versus the placebo-treated group. There were very few AEs overall, and the number of events did not increase in a dose-dependent manner (Table 2). The majority of AEs occurred in only 1 subject in any treatment group. Headache was the only event that occurred in 2 subjects in any treatment group. No events occurred in >2 subjects in any treatment group. Toreforant was not associated with any significant effects on laboratory parameters, vital signs, or physical examination. Two subjects (1 placebo-treated subject, 1 toreforant 150 mg-treated subject) had an increase in the QT interval corrected for heart rate using the Fredericia formula (QTcF) from baseline of 30 to 60 msec. The longest QTcF value after treatment was 451 msec, which occurred in 1 subject on the 150 mg dose. No subject receiving toreforant had an increase from baseline in QTcF >60 msec or an absolute QTcF value >500 msec.

Toreforant was well tolerated when administered orally at doses of up to 300 mg daily in healthy male subjects and 100 mg daily in healthy female subjects for 14 consecutive days. No deaths, serious adverse events, or discontinuations due to AEs were reported. Gastrointestinal disorders and musculoskeletal disorders occurred more commonly in subjects receiving toreforant compared to placebo; however, the incidence of AEs did not occur in a consistently dose-dependent manner (Table 3). All the AEs were mild or moderate in severity except one. Severe treatment-emergent pruritus was observed in 1 subject after administration of 150 mg toreforant and was accompanied by moderate erythematous rash. Toreforant was not associated with any significant effects on vital signs or physical examination. Mild increases in serum creatinine were observed in a dose-dependent manner at dosages up to 300 mg/day. This is most likely due to the inhibition of the organic cation transporter 2 and multidrug and toxin extrusion-1 and -2K (data not shown). Dose-dependent effects on QTcF were observed with toreforant at doses of 100 mg once daily and 300 mg once daily in male subjects. None of the subjects had an increase of greater than 60 msec from baseline in QTcF. There were 6 subjects with an increase between 30 msec and 60 msec from baseline in the QTcF (1 [7%] placebo; 2 [29%] 100 mg male; and 3 [43%] 300 mg). No subject experienced absolute QTcF of greater than 450 msec at any time point after baseline. No arrhythmias were reported during the 24-hour telemetry monitoring on Days 1, 7, and 14.

Following the administration of single or multiple oral doses in healthy adult subjects under fasting conditions, toreforant was rapidly absorbed with a median Tmax ranging from 1 to 4 hours postdose and extensively distributed to tissues as evidenced by the large apparent volume of distribution after extravascular administration (Vd/F) (ie, Vd/F >1,000 L). Toreforant elimination appeared to involve both liver metabolism and renal excretion with a mean terminal t1/2 increasing with dose and ranging from 56 to 94 hours (Supplemental Tables S4 and S5). The Cmax and AUC of toreforant increased more than dose proportionally across the dose range from 5 to 150 mg after single dose

### Table 3: Treatment-emergent adverse events with an incidence of >1 in treatment groups after 14 days of oral dosing of toreforant.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N = 15)</th>
<th>15 mg (N = 7)</th>
<th>50 mg (N = 7)</th>
<th>100 mg Male (N = 7)</th>
<th>100 mg Female (N = 7)</th>
<th>300 mg (N = 7)</th>
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<tr>
<td></td>
<td>n (%)</td>
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<td>n (%)</td>
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<tr>
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<td>2 (29)</td>
<td>0</td>
<td>3 (43)</td>
<td>1 (14)</td>
<td>5 (71)</td>
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<td>0</td>
<td>4 (57)</td>
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<td>1 (14)</td>
<td>1 (14)</td>
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<td>2 (29)</td>
<td>0</td>
<td>2 (29)</td>
<td>1 (14)</td>
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<tr>
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<td>0</td>
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<td>0</td>
<td>2 (29)</td>
<td>1 (14)</td>
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</tr>
<tr>
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<tr>
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<td>4 (57)</td>
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<td>4 (57)</td>
<td>1 (14)</td>
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Figure 7: Plasma concentration of toreforant (mean ± standard deviation) at various time points after oral doses on days 1, 7 and 14 in the multiple dose portion of the clinical study. Doses were 15 mg males (♦), 50 mg males (□), 100 mg males (●), 100 mg females (○) and 300 mg males (▼).

Figure 8: Clinical ex vivo histamine-induced eosinophil shape change. Blood was stimulated with 0.3 μM histamine for 10 min and then the forward scattering was assessed by flow cytometry. The percent change in the geometric mean of the forward scatter observed when blood from dosed subjects was stimulated with 0.3 μM histamine compared to samples where no histamine was added is plotted as box-and-whisker plots indicating the maximum and minimum response. Data from the multiple ascending dose clinical study is shown. Data were collected 1 day prior to dosing on day 1 (-24 h) and at several time points after dosing on day 14. Statistical comparison of treated groups to the placebo group was assessed by a one-way ANOVA with post-hoc Dunnett’s test. *p < 0.05.

administration and across the dose range from 15 to 300 mg after once daily multiple-dose administration for 14 consecutive days (Figure 6 and 7). Toreforant exposure appeared to reach steady-state by Day 14 for doses up to 100 mg once daily. The accumulation ratio at steady state based on AUC_{0-24} was approximately 4 to 6, corresponding to an effective t_{1/2} ranging from 57 to 95 hours. At the dose of 100 mg once daily, plasma exposures of toreforant (both C_{min} and AUC_{0-24}) were approximately 20% higher in female subjects than male subjects on both Day 1 and Day 14.

Histamine-induced eosinophil shape change was used as a pharmacodynamic readout in both phase 1 studies. In the SAD study, a trend of inhibiting histamine-induced eosinophil shape change at 1 and 4 hours post dose in the 150 mg cohorts was observed (data not shown). For the MAD study, samples were collected h prior to dosing on Day 1 and on days 1, 7 and 14 (1.5, 4, and 24 - h post dose). At baseline all treatment groups had a similar percentage change in mean forward scatter upon histamine stimulation. Statistically significant histamine-induced shape change inhibition was observed in the 50, 100 and 300 mg cohorts at 1.5, 4 and 24 h after the last dose on Day 14, but not in placebo and the 15 mg groups (Figure 8). At 24 hours post Day 14 dose approximately 40, 30 and 75% inhibition were observed for the 50, 100 and 300 mg, cohorts, respectively. The results from the female subjects given 100 mg were similar to the results from males (data not shown).

Discussion

Toreforant is one of the few H4R ligands to enter clinical studies. Indeed, it was most likely the first selective H4R antagonists to be tested in the clinic as the first phase 1 study started in January 2008. Toreforant is a potent and selectivity H4R ligand with the affinity for the human H4R being 25-fold higher than for the histamine H3 receptor and no detectable affinity for the histamine H1 or H2 receptors. The compound also exhibits antagonist properties at the H4R of various species tested and in primary cell assays. However, toreforant has relatively low affinity for the mouse H4R that is only 4-5-fold higher than at the mouse histamine H3 receptor and 13- fold higher than the mouse histamine H2 receptor. Therefore, in mouse models high doses are needed to achieve activity and one must be cautious about the contributions of the other receptors at these doses. Nevertheless, toreforant does behave in the mouse asthma and collagen-induced arthritis models similar to other reported H4R antagonists such as JNJ 7777120, JNJ 28307474 and JNJ 39758979 [6,12,13,20].

In particular, toreforant showed activity in a mouse collagen-induced arthritis model. In this model experience with other H4R antagonists indicated that doses that yielded trough values above the mouse H4R Ki were efficacious, whereas lower doses were not. This is consistent with the dose response observed with toreforant where the 100 mg/kg twice daily dose yielded a C_{min} of 550 nM close to the mouse H4R Ki of 307 nM. The 50 mg/kg twice daily dose was not efficacious and the trough concentrations are below this threshold. This mouse model has been used to predict efficacy in rheumatoid arthritis (RA) in humans and efficacy of toreforant in this model was the rationale for testing the compound in two clinical rheumatoid arthritis studies [7]. In the phase 2a proof-of-concept study, treatment with toreforant 100 mg/day for 12 weeks improved signs and symptoms of RA in a small population of RA patients (ACR20 response of 48% for patients on toreforant and 14% for those on placebo), although the study was terminated early and the analysis was post hoc. However, a subsequent phase 2b dose ranging study, treatment with toreforant at 3, 10 and 30 mg/day for 24 weeks did not show any efficacy relative to placebo (ACR20 response of 37%, 36%, 47% and 37% for placebo, 3 mg, 10 mg and 30 mg groups, respectively). It is possible that the discrepancy in the results between the two studies could be due to the post-hoc nature of the analysis in the phase 2a study. Another explanation could be that toreforant may have only minimal efficacy in RA that was detected in the phase 2a study due to an unusually low placebo effect [7]. A low level of efficacy for toreforant may be predicted by the preclinical arthritis model used. Toreforant showed efficacy in a mouse collagen-induced arthritis model that is known to be highly IL-17 dependent. Indeed H4R antagonists have been shown to modulate IL-17 responses in this model [13]. However, clinical studies with antibodies that target this cytokine or its receptor have little, if any, efficacy in rheumatoid arthritis [21].

It is also possible that toreforant is efficacious at the higher dose, 100 mg/day, but not at lower doses, although the data from animal model suggested that the exposure at lower doses still exceeded the required
minimal efficacious level. If one takes into account the difference in the $K_v$ values and $pA_2$ values between the human and mouse receptors, the $C_{mic}$ value at 100 mg/kg in the mouse model would translate into the $Ki$ values and $pA_2$ values between the human and mouse receptors, minimal efficacious level. If one takes into account the difference in species or strain used and the sensory modalities used to test the response. To this point it is somewhat striking and unexpected that the doses in the second study 3, 10 and 30 mg, should not have worked since there would be little or no inhibition in the eosinophil assay at these doses. However, toreforant is highly protein bound and highly tissue distributed and therefore one could question whether target engagement, i.e. inhibition of the eosinophil shape change, in blood has any relevance to that in tissues. Ideally, one would measure the pharmacodynamic activity in animals at doses of the compound that yield efficacy in the disease models and then use this to predict what level of pharmacodynamic activity would be needed for efficacy in humans. Unfortunately, this assay cannot be conducted in mice for several technical reasons. However, the human data either in vitro or from the phase 1 clinical studies are very consistent with the in vitro pharmacology at the receptor and, therefore, one can use the $K_v$ value at the receptor. The $EC_{mic}$ and concentration of the agonist, histamine, in combination with the amount of protein binding (since the assay is in whole blood only the free fraction of compound should bind the receptor) to estimate the expected percent inhibition of the histamine-induced eosinophil shape change. For the human pharmacodynamics assay the predicted results align quite well with the observed results for both toreforant and JNJ 39758979 [6]. Therefore, this same approach can be used to estimate the level of eosinophil shape change that the 100 mg/kg efficacious dose would yield if indeed it could be conducted. The free concentration of toreforant 24 h after the 100 mg/kg dose would be approximately 70 nM and this would translate into approximately 10% inhibition in the eosinophil shape change assay given the mouse $K_v$ of ~300 nM. Therefore, the animal data predict that for toreforant the dose needed for efficacy in humans.

Conclusion

Toreforant is a potent and selective H4R antagonist. It has a preclinical efficacy profile similar to other H4R antagonists except that it does not have activity in pain and pruritus models, perhaps related to lack of CNS exposure. Toreforant was safe and well tolerated in preclinical toxicology studies and in phase 1 study in healthy subjects. This combined with excellent oral pharmacokinetics makes it a useful compound for studying the efficacy of H4R antagonists in humans.

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Authorship Contributions and Disclosures

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

Disclosures: All authors are current or former employees of Janssen Research & Development, LLC. And own stock in Johnson & Johnson.

References

2. Dunford PJ, Williams KN, Desai PJ, Karlsson L, McQueen D, Thurmond RL. Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. J Allergy Clin


