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ARTICLE

Pharmacokinetics, pharmacodynamics, and safety of izuforant, an H4R inhibitor, in healthy subjects: A phase I single and multiple ascending dose study

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Abstract

Izuforant is a selective, and potent histamine H4 receptor (H4R) antagonist developed to treat atopic dermatitis (AD). There is an unmet medical need for therapeutic agents to control inflammation and pruritus. Izuforant is a strong candidate for this task based on the findings of non-clinical studies showing that inhibition of the histamine-mediated signaling pathway via H4R by izuforant results in decreased pruritus and inflammation. This study aimed to evaluate the clinical pharmacokinetic (PK) and pharmacodynamic (PD) profiles of izuforant. Dose-block-randomized, double-blind, placebo-controlled, single- and multiple ascending dose studies were conducted in 64 healthy volunteers. For the single ascending dose (SAD) study, 10–600 mg izuforant was administered to the designated groups. For the multiple ascending dose (MAD) study, 100–400 mg izuforant was administered to three groups. The clinical pharmacokinetic (PK) profile of izuforant was evaluated using plasma and urine concentrations. Blood sampling for the PD assay, which measured imetit-induced eosinophil shape changes (ESC), was also conducted. A one-compartment PK model described the distribution and elimination profiles of izuforant. An imetit-induced ESC inhibition test was established and validated for PD evaluation as a measure of the H4R antagonistic effect. ESC inhibition was observed even at doses as low as 10 mg; however, this inhibition became stronger and lasted longer as the dose increased. All izuforant doses were well tolerated, and no discontinuations due to adverse events (AE) or deaths were reported.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Izuforant is a selective, and potent histamine H4 receptor (H4R) antagonist developed to treat atopic dermatitis (AD). Inhibition of the histamine-mediated

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signaling pathway through H4R, as observed in non-clinical studies of izuforant, controls both pruritus and inflammation. Currently, in clinical settings, there is an unmet medical need for a therapeutic agent that can control both inflammation and pruritus. Izuforant is considered a strong candidate for this task.

WHAT QUESTION DID THIS STUDY ADDRESS?

This first-in-human study assessed the safety, tolerability, pharmacokinetics, and pharmacodynamics of izuforant administered as either a single or multiple ascending doses in healthy Korean volunteers, compared with placebo.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

All izuforant doses tested were safe and well tolerated by the study participants. Linear kinetics best described the PK profile of izuforant. izuforant inhibited imetit-induced eosinophil shape changes, resulting in full inhibition lasting over 24 h at high doses, suggesting that izuforant is a potent H4R antagonist. The clinical PK and PD characteristics of izuforant suggest that the drug could be used as a once-daily dosing regimen in further clinical studies.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Our study, in addition to a non-clinical study, may accelerate further development of izuforant for treating patients with AD.

INTRODUCTION

Izuforant (JW Pharmaceutical Co. Gyeonggi-do, Korea) is a potent histamine H4 receptor (H4R) antagonist newly developed for oral administration in the treatment of atopic dermatitis (AD). Histamine is mainly involved in the pathophysiology of allergic diseases, such as asthma, allergic rhinitis, and AD, in regulating T helper lymphocytes, and mediating mast cell activation.[1] H4R is coupled to Gα/io proteins and is expressed by various immune cells, especially antigen-presenting and Th2-type T cells involved in allergic immune responses.[2–10]

The stimulation of H4R by chemokines results in the chemotaxis of leukocytes to sites of inflammation, mediating the histamine response.[11,12] Histamine enhances adhesion molecule expression, cell shape changes, and cytoskeletal rearrangement via H4R, leading to increased eosinophil migration. Eosinophils are inflammatory cells whose immune regulatory functions are modulated by histamine. Changes in eosinophils' shape result in the cytoskeleton's formation, providing directionality and force for cellular migration.[13,14] Furthermore, histamine-induced H4R stimulation increases mast cell recruitment and induces immune response amplification and chronic inflammation.[1]

Pruritus is a common clinical symptom related to skin diseases such as AD.[15,16] Among the mediators of pruritus in AD, histamine induces pruritus by binding to histamine receptors on sensory nerve fibers.[17] However, cytokines and neuropeptides other than histamines also affect pruritus in AD; consequently, pruritus in AD is difficult to resolve with antihistamines.[18,19]

Atopic dermatitis is a common chronic inflammatory skin disease accompanied by intense pruritus and unsightly lesions.[20] Scratching and persistent itchiness, the main clinical symptoms of AD, reduce skin barrier function and impair patients' quality of life.[20–22] There is a lack of effective systemic treatment options to control chronic symptoms in patients with moderate-to-severe ADs.[23–25] Therefore, there is an unmet medical need for therapeutic agents that can effectively relieve both inflammation and pruritus in clinical settings.

In our earlier work, izuforant showed selective binding affinity to H4R and concentration-dependent inhibitory effects on eosinophil shape changes (ESC) induced by imetit, which mimics the histamine effect in triggering eosinophil changes and has an agonistic effect on H4R in the human whole-blood assay with IC50 of 65.1–72.2 nM.[13] The affinity (pKi) of izuforant at mouse and rat histamine receptor subtypes was 6.83 and 5.36 for H4R, with lower pKi values observed for other histamine receptor (unpublished data). Inhibitory effects were also observed in an antigen-specific Th2 type immune response, subsequently reducing Th2 cytokine-related skin inflammation and dermal thickness in the ear in a mouse model of epicutaneous sensitization and oxazolone-mediated AD (unpublished data). Izuforant also produced markedly lower mean scratching behavior when orally administered in a murine model of IgE-mediated pruritus compared with other antihistamines (unpublished data).

Therefore, izuforant has the potential to effectively control inflammation and pruritus. This study aimed to

explore the clinical pharmacokinetic and pharmacodynamic characteristics of izuforant and the safety and tolerability profiles of izuforant administered to healthy adult volunteers in single ascending dose (SAD) and multiple ascending dose (MAD) studies.

METHODS

Study population

Healthy male Korean subjects aged 19–55 years with body mass indices between 18.5 and 28.0 kg/m^2 were enrolled in this study. After voluntarily signing the informed consent form, the volunteers underwent the following screening procedures: medical history, physical examination, 12-lead electrocardiogram, and assessment of clinical laboratory parameters, including hematology, blood chemistry, and urine drug screening.

Study design, treatments, and administration

Dose-block-randomized, double-blind, placebo-controlled SAD and MAD studies were performed. In the SAD study, the dose was increased in five steps (10, 30, 100, 300, and 600 mg) from the lowest to the highest dose. Three cohorts treated with different dose levels (100, 200, and 400 mg) were included in the MAD study. The dose levels used in the MAD study were determined after analysis of both pharmacokinetic (PK)/pharmacodynamic (PD) and safety data from the SAD study. The starting dose (10 mg) of the SAD study was selected based on the calculated safety margin derived from the no observed adverse effect level (NOAEL) of the non-clinical toxicology studies and the pharmacologically active dose (PAD) in non-clinical pharmacology studies.[26]

Each dose group consisted of eight subjects (six receiving izuforant and two receiving the placebo). A sentinel group (two subjects, one subject receiving izuforant and the other subject receiving placebo) was separated from the remaining six subjects for each dose group in the SAD study to confirm the safety of the first administration of izuforant in humans.

Safety evaluations were conducted by the Safety Review Committee (SRC), to confirm that no safety problems were identified in each dose group of the SAD study. A MAD study was also conducted after the SRC reviewed the safety and tolerability results of the SAD study. A single dose of izuforant or placebo was orally administered to randomly assigned participants in the SAD study with fasted state. Izuforant or placebo was orally administered once daily for 7 days in the MAD study with a fasted state.

In the SAD study, blood sampling for PK assessment were conducted pre-dose (0 h) and 0.25, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 48, and 72 h post-dose. In the MAD study, blood PK sampling was conducted pre-dosing on days 1 to 7 (0 h) and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 h post-dosing on days 1 and 7. Post-dose PK sampling was also conducted at 24, 48, and 72 h following the final dose.

Blood samples for PD assessment were taken 24 h before the first administration of izuforant on day 1 (pre-dose) and at 1, 3, 6, 24, 48, and 72 h post-dose. The temporary analysis results for PK, PD, and safety in the SAD study were also considered, and the blood PD sampling time points in the MAD study were set to 1, 6, and 24 h post-dosing on day 1 and 1, 6, 24, 48, and 72 h post-dosing on day 7.

Voided urine samples were collected for PK assessment pre-dosing (0 h) and from 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 h post-dosing in the SAD study. The urine collection time intervals in the MAD study were pre-dose (0 h), and from 0 to 4 h, 4 to 8 h, 8 to 12 h, and 12 to 24 h post-dose, on day 1. Urine was collected on day 7 at 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 h post-dosing. Urine collection in the MAD study was performed only in the 400 mg dose group.

Measurement of plasma and urine izuforant concentration

The plasma and urine concentrations of izuforant were measured using a validated ultra-performance liquid chromatography assay with a Waters ACQUITY UPLC system (Waters, Milford, MA, USA) coupled with a Waters Xevo TQ MS system (Waters, Milford, MA, USA).

The calibration curve was linear over a range of 10–10,000 ng/mL, with the correlation coefficient (r) greater than 0.995. The inter-day and intra-day accuracies were within 85%–115%. The coefficient of variability representing the overall precision was below 20% for the lower limit of quantification and 15% for the upper concentration range of the calibration curve.

Imetit-induced eosinophil shape change assay

At the time points specified, venous blood samples (490 μL each) were collected into heparin tubes. The samples were treated with a negative control (no treatment) or

50 nM imetit dihydrobromide (PubChem ID: 11957573) for 5 min at 37°C to measure the eosinophil shape change (ESC). To measure ESC, a gated autofluorescence forward scatter assay was performed using a BD FACS II system. Scans treated with or without imetit were summarized by calculating the average of the geometric means of forward scatter (FSC). The eosinophil population was gated using a validated method.[13] ESC (%) was calculated as follows:

$$\text{ESC}(\%) = \frac{\text{Geometric mean of FSC}_i - \text{Geometric mean of FSC}_b}{\text{Geometric mean of FSC}_b} \times 100$$

The geometric mean of FSC_i was the mean scatter of eosinophils treated with 50 nM imetit, and the geometric mean of FSC_b was the mean scatter of eosinophils treated with the negative control (no inhibitor).

Safety assessment

The safety and tolerability were assessed throughout the study. To evaluate safety, all adverse events (AEs) were monitored based on physical examination, vital signs, 12-lead ECG, routine hematology, serum chemistry, and urinalysis. All AEs reported by the subjects or detected in the assessments were recorded; the investigators determined their relationship with the treatment.

Pharmacokinetic analysis

WinNonlin (Ver 8.2, Pharsight, NJ, USA) was used for PK parameter calculations and non-compartmental analysis. AUC during a dosage interval (τ) ($AUC_{\tau(,ss)}$) on days 1 and 7, AUC from zero to time of last measurable concentration (AUC_{last}) and AUC from zero to infinity ($AUC_{inf(,ss)}$) were calculated. Terminal elimination rate constant (λ_z) was estimated using the linear regression analysis on the terminal portion of the log-transformed plasma concentration-to-time profile. The elimination half-life ($t_{1/2}$), apparent clearance ($CL_{(,ss)}/F$), apparent volume of distribution ($Vd_{(,ss)}/F$), AUC of the last dosing time extrapolated to infinity ($AUC_{inf(,ss)}$), and renal clearance ($CL_{R(,ss)}$) were calculated. $CL_{R(,ss)}$ was calculated using the following formula where A_e/dose denotes the amount of the drug excreted unchanged in urine divided by the dose.

$$CL_{R(,ss)} = \frac{A_e}{\text{Dose}} \times \frac{CL_{(,ss)}}{F}$$

Pharmacodynamic analysis

The percentage of ESC inhibition from baseline activity was calculated. The PD parameters of izuforant, which used the percentage inhibition of ESC from baseline activity over time, were calculated by non-compartmental analysis using WinNonlin (Ver. 8.2, Pharsight, NJ, USA). The $E_{max(,ss)}$ and $T_{max(,ss)}$ values of izuforant were obtained from the observed values. The area under the effect curve (AUEC) below the baseline was calculated using the linear trapezoidal rule.

Statistical analysis

Descriptive statistics of subject demographics are presented for each study. An analysis of variance (ANOVA) test or the Kruskal–Wallis test was used to compare more than two treatment groups. All statistical analyses were performed using SAS (version 9.4; Cary, NC, USA) or WinNonlin (version 8.2; Pharsight, NJ, USA). Statistical significance was set at $p < 0.05$.

The PK data were described as the mean ± standard deviation (SD) in each dose group, except for T_{max}, where the PK data were presented using the median (range). The dose proportionality of the PK parameters (C_{max}, AUC_{last} and AUC_{inf} for the SAD study and $C_{max,ss}$ and $AUC_{\tau,ss}$ for the MAD study) in the SAD study and the MAD study was explored using the power model.[27]

For ESC, a repeated-measures mixed model was implemented to compare the differences in the averages between the dose and placebo groups at each measurement timepoint. Dose group, time, and interaction (dose group × time) were incorporated as fixed effects, and the subject was considered to have a random effect in the repeated-measures mixed model. Furthermore, ESC 24 h before the first IP administration to each subject was used as a covariate for adjustment in the model. Dunnett's adjustment was used to perform multiple comparisons of the difference between the least-squares mean values for each dose group and the placebo group at each measurement timepoint.

Ethics

This study was approved by the Institutional Review Board (IRB) of Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea (IRB number: 4-2018-1098), and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. All volunteers were enrolled in the study after providing written informed consent.

RESULTS

Demographics

Sixty-three (63) subjects completed the studies (SAD study: 40, MAD study: 23). One subject in the MAD study (400 mg dose group) had to be withdrawn from the study on day 6 owing to AE and mild nausea and vomiting. The subject was included in the demographic and safety populations but was excluded from the PK/PD analysis population. There were no statistically significant differences in age, height, body weight, or BMI between the dose groups in any of the studies, except for weight in the MAD study; the weight difference was clinically insignificant (Table S1).

Pharmacokinetics

The mean plasma concentration–time profile and the mean plasma and urine PK parameters of izuforant according to dose in the SAD study are presented in Figure 1 and Table 1, respectively. The corresponding results of izuforant in MAD study are summarized in Figure 2 and Table 2, respectively.

Izuforant showed rapid absorption with a median $T_{max(,ss)}$ of 1.00–1.75 h. The C_{max} in the SAD study, $C_{max,ss}$ in the MAD study increased with dose, with peak values of 4495 ng/mL and 3630 ng/mL, respectively. The AUC_{inf} in the SAD study and the $AUC_{inf,ss}$ in the MAD study increased with dose, with peak values of 40,847 and 27,487 ng/mL, respectively. Vd/F, a parameter of drug disposition, was also consistent across all dose groups. The calculated range of $t_{1/2}$ of izuforant in the SAD study was between 2.42 h and 7.80 h. The $t_{1/2}$ values of izuforant in the 10 mg and 30 mg of the SAD study were shorter (2.42 h in the 10 mg dose group and 4.84 h in the 30 mg dose group) than the values of other dose groups in the SAD and the MAD study that ranged between 6.36 h and 7.80 h. The fraction of izuforant excreted unchanged in the urine, given as the ratio of the total amount of izuforant excreted unchanged to the dose of izuforant, was 42%–77%. The fluctuation (peak-to-trough ratio) at the steady state was consistent among all dose groups, with an accumulation index (AI) of 1.04–1.39, indicating that an accumulation of izuforant in repeated dosing could be clinically neglected.

Dose proportionality of the log-transformed PK parameters was evaluated using a power model. In the case of the SAD study, with a dose range of 10–600 mg, the β-values and 90% CIs of C_{max}, AUC_{last} and AUC_{inf} did not fall into the acceptable range, except for the lower limit of the 90% CI of C_{max} (the β value [with 90% CI] for C_{max} was 1.08 [1.03–1.13], for AUC_{last} was 1.28 [1.23–1.32], for AUC_{inf} was 1.26 [1.22–1.31], and the acceptable range for β-values was 0.95–1.05). Based on these results, the increase in drug exposure following a single dose was judged to be greater than or proportional to the dose.

The 90% CI of $C_{max,ss}$ and $AUC_{\tau,ss}$ in the MAD study with 100–400 mg of dose range included 1, but the CIs did not fall into the acceptable range (the β value [90% CI] for $C_{max,ss}$ was 0.97 [0.83–1.12], for $AUC_{\tau,ss}$ was 0.97 [0.80–1.13], and the acceptable range for β-values, was 0.84–1.16).

Pharmacodynamics

Figure 3 shows the results of the statistical comparison of imetit-induced ESC in the SAD and MAD studies. Figure S1 shows the results of the change from baseline of imetit-induced ESC by Dose Group in the SAD and MAD studies. Table S2 presents the results of the non-compartmental PD parameter analysis of the SAD and MAD studies. There was no statistical difference in baseline imetit-induced ESC (24 h prior to the first dose) in each dose group or in the placebo group in either the SAD or MAD studies.

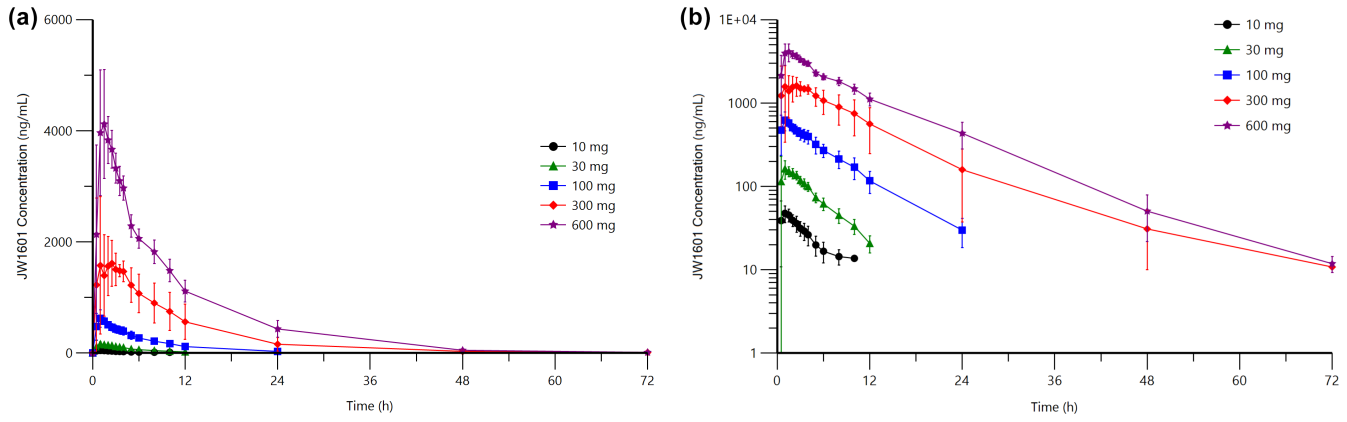

FIGURE 1 Mean plasma concentration–time profiles of izuforant after single administration. Error bars denote the standard deviation. (a): Linear scale; (b): Semi-log scale.

TABLE 1 Pharmacokinetic parameters for izuforant after administration of single oral dose in healthy subjects.

	Dose group				
Parameters	**10 mg (*n* = 6)**	**30 mg (*n* = 6)**	**100 mg (*n* = 6)**	**300 mg (*n* = 6)**	**600 mg (*n* = 6)**
T_{max} (h)	1.0 (0.50–1.50)	1.25 (0.50–2.00)	1.00 (0.50–1.50)	1.75 (0.50–5.00)	1.50 (1.00–2.50)
C_{max} (ng/mL)	56.6 ± 10.5	192.7 ± 55.9	701.5 ± 74.0	2537 ± 1055	4495 ± 756
AUC_{las} (h*ng/mL)	215.9 ± 64.4	1012 ± 119	4728 ± 784	18,253 ± 5843	40,603 ± 5849
AUC_{inf} (h*ng/mL)	233.4 ± 69.4	1047 ± 118	4779 ± 785	18,330 ± 5888	40,847 ± 5920
$t_{1/2}$ (h)	2.42 ± 0.81	4.84 ± 0.37	7.05 ± 0.37	6.36 ± 1.27	7.80 ± 1.20
CL/F (L/h)	46.0 ± 12.7	29.0 ± 3.4	21.5 ± 4.1	17.5 ± 4.5	15.0 ± 2.3
Vd/F (L)	150.4 ± 31.1	203.0 ± 34.4	218.0 ± 39.0	154.6 ± 20.2	165.5 ± 13.1
A_e (mg)	4.16 ± 0.61	15.6 ± 1.1	68.0 ± 5.3	196.6 ± 51.3	388.6 ± 64.1
CL_R (L/h)	18.5 ± 3.1	15.1 ± 2.3	14.5 ± 1.9	11.3 ± 3.7	9.55 ± 1.35

Note: Data are summarized as arithmetic mean ± standard deviation values except those for T_{max}, for which the median (min–max) values are presented.

Abbreviations: A_e, amount of drug excreted unchanged in urine; AUC_{inf}, area under the plasma drug concentration–time curve from dosing time extrapolated to infinity; AUC_{last}, area under the plasma drug concentration–time curve from the time of dosing to the last measurable concentration; CL/F, apparent clearance; CL_R, renal clearance; C_{max}, maximum concentration of the drug in plasma; $t_{1/2}$, elimination half-life; T_{max}, time to C_{max}; Vd/F, apparent volume of distribution.

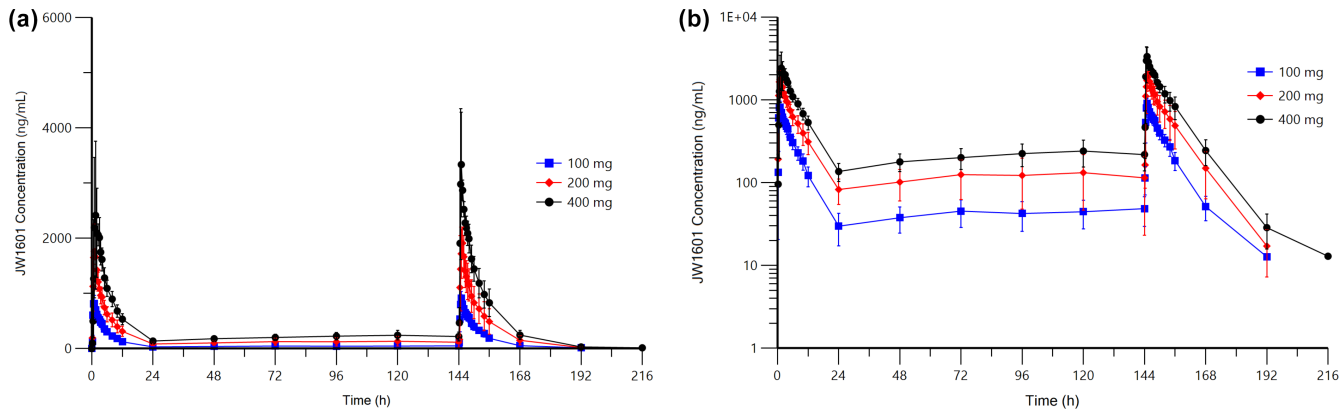

FIGURE 2 Mean plasma concentration–time profiles of izuforant after multiple administrations. Error bars denote the standard deviation. (a): Linear scale; (b): Semi-log scale.

In the SAD study, statistically significant differences in imetit-induced ESC inhibition and the placebo group were maintained for 6 h in the 10 mg cohort and 24 h in the other cohorts at each corresponding timepoint. In the MAD study, statistically significant differences in imetit-induced ESC inhibition and the placebo group were maintained for 1 h in the 100 mg cohort and for 24 h in other cohorts from the last dosing, at each corresponding timepoint. However, statistically significant imetit-induced ESC inhibition 6 h after the last drug dose in the 400 mg dose group was not observed.

Safety assessment

In the SAD study, 18 AEs were reported in 13 subjects (32.5%): two subjects (20.0%) in the placebo group, two subjects (33.3%) in the 10 mg dose group, one subject (16.7%) in the 30 mg dose group, one subject (16.7%) in the 100 mg dose group, one subject (16.7%) in the 300 mg dose group, and six subjects (100.0%) in the 600 mg dose group (Table 3). According to the classification of AEs defined in CTCAE version 5.0, there were three grade 4 AEs observed in the SAD study (neutrophil count decreased in the placebo group, blood creatine phosphokinase increased in the 30 mg dose group, and hypertriglyceridemia in the 600 mg dose group), and one grade 3 AE (syncope in the 600 mg dose group). Other AEs were classified as grade 1 or grade 2 and no grade 5 adverse events were reported. Although one serious AE (SAE) (gastrointestinal hemorrhage leading to hospitalization) occurred in the 10 mg dose group, its relationship with IP was unlikely because the onset was 6 days post-dosing and the subject had a history

TABLE 2 Pharmacokinetic parameters for izuforant after administration of multiple oral dose in healthy subjects.

Parameters	Dose group		
	100 mg ($n=6$)	200 mg ($n=6$)	400 mg ($n=5$)
Day 1			
T_{max} (h)	0.75 (0.50–1.50)	1.00 (0.75–2.00)	1.50 (1.00–3.00)
C_{max} (ng/mL)	896.3 ± 203.9	1980 ± 216	2794 ± 521
$AUC_{0\text{-}24h}$ (h*ng/mL)	4972 ± 779	11,120 ± 2052	17,691 ± 2389
A_e (mg)	–	–	204.8 ± 84.6
Day 7			
$T_{max,ss}$ (h)	1.00 (0.75–1.50)	1.25 (0.50–2.00)	1.00 (0.75–1.50)
$C_{max,ss}$ (ng/mL)	935.3 ± 105.1	2217 ± 316	3630 ± 855
$C_{min,ss}$ (ng/mL)	46.6 ± 17.5	113.3 ± 89.0	217.8 ± 79.6
$C_{avg,ss}$ (ng/mL)	269.5 ± 40.2	610.0 ± 189.9	1029.7 ± 206.9
$AUC_{\tau,ss}$ (h*ng/mL)	6469 ± 966	14,640 ± 4558	24,712 ± 4965
$AUC_{inf,ss}$ (h*ng/mL)	7047 ± 1144	16,202 ± 5504	27,487 ± 6018
$t_{1/2}$ (h)	7.07 ± 0.69	7.00 ± 0.58	7.52 ± 0.88
CL_{ss}/F (L/h)	15.76 ± 2.42	14.56 ± 3.56	16.69 ± 3.14
Vd_{ss}/F (L)	161.4 ± 34.4	145.5 ± 31.1	179.2 ± 27.8
AI ($C_{max,ss}$)	1.08 ± 0.22	1.12 ± 0.14	1.22 ± 0.16
AI ($AUC_{\tau,ss}$)	1.31 ± 0.14	1.30 ± 0.18	1.39 ± 0.12
A_e (mg)	–	–	306.6 ± 113.3
$CL_{R,ss}$ (L/h)	–	–	12.96 ± 5.46

Note: Data are summarized as arithmetic mean ± standard deviation values except those for $T_{max(,ss)}$ for which the median (min–max) values are presented.

Abbreviations: A_e, amount of drug excreted unchanged in urine; AI, accumulation index; AUC_{0-24h}, area under the plasma drug concentration–time curve from the time of dosing to the 24 h after drug administration; $AUC_{inf,ss}$, area under the plasma drug concentration–time curve extrapolated to infinity at steady state; $AUC_{\tau,ss}$, area under the plasma drug concentration–time curve within a dosing interval (τ) at steady state; $C_{avg,ss}$, average concentration of drug in plasma at steady state; $CL_{R,ss}$, renal clearance at steady state; CL_{ss}/F, apparent clearance at steady state; C_{max}, the maximum concentration of the drug; $C_{max,ss}$, maximum concentration of drug in plasma at steady state; $C_{min,ss}$, minimum concentration of drug in plasma at steady state; $t_{1/2}$, terminal elimination half-life; T_{max}, time to C_{max}; $T_{max,ss}$, time to $C_{max,ss}$; Vd_{ss}/F, apparent volume of distribution at steady state.

of similar symptoms. The SAE was resolved without complications. Seventeen AEs were reported as adverse drug reactions (ADRs).

In the MAD study, 35 AEs occurred in 10 subjects (41.7%): one subject (16.7%) in the placebo group, one subject (16.7%) in the 100 mg dose group, three subjects (50.0%) in the 200 mg dose group, and five subjects (83.3%) in the 400 mg dose group (Table 4). According to the classification of AEs defined in CTCAE version 5.0, All AEs observed in the MAD study were classified as grade 1 or grade 2, and no grade 5 adverse events were reported. All AEs were reported as ADRs.

There were no clinically significant drug-related AEs in this study, and all subjects recovered without any sequelae despite tolerability issues in the highest dose groups in the SAD and MAD studies. All AEs that occurred for both studies are summarized in Tables S3 and S4.

DISCUSSION

This study is the first one to reveal the PK, PD, and safety of the H4 receptor antagonist, izuforant, in healthy Korean subjects. In this study, izuforant showed a first-order elimination profile solely based on the semi-log PK profile of izuforant, with $t_{1/2}$ in the range of 2–8 h. Therefore, a one-compartment PK model with first-order absorption may describe the PK of izuforant. The fraction of izuforant excreted unchanged in the urine was more than 50%, except in the 10 mg dose group in the SAD study. This is consistent with the known elimination pathway of izuforant (unpublished data). The results of the MAD study suggest the possibility of dose-proportional PK. In this study, izuforant potently inhibited the imetit-induced ESC profile, suggesting a sustained effect for a substantial duration. Iimetit-induced ESCs were inhibited for more than 24 h in groups administered a dose of at least 200 mg.

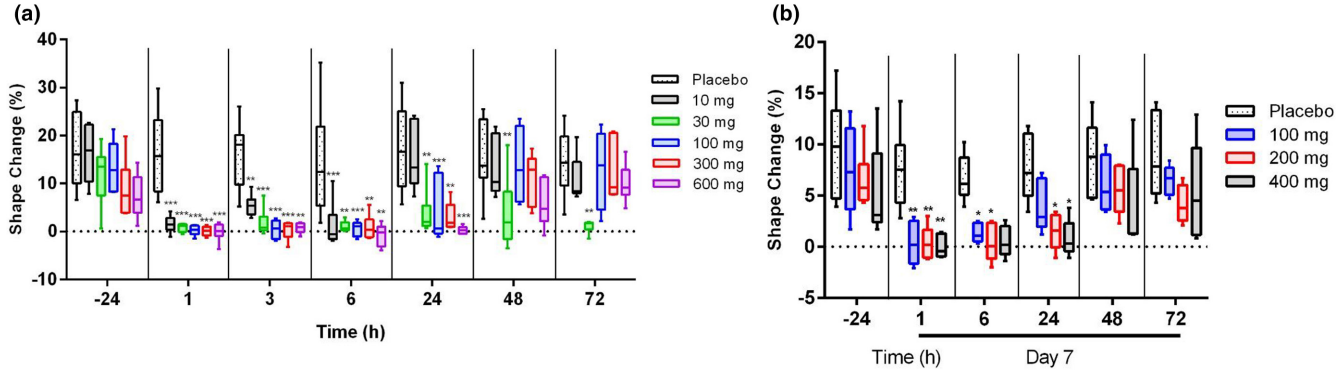

FIGURE 3 Box-and-whisker plots indicating the maximum and minimum imetit-induced ESC by Dose Group, (a) single and (b) multiple ascending oral dose studies. Statistical comparison of treatment groups to the placebo group was assessed by repeated-measures mixed model with post hoc Dunnett's adjustment. **p*-value <0.05, ***p*-value <0.01, ****p*-value <0.001.

The AUC_{last} and AUC_{inf} values were higher than those of the proportional dose. The decrease in izuforant clearance with dose escalation in the SAD study may have affected the dose-dependent increase in izuforant exposure. As izuforant is excreted mainly via the renal route, a saturable renal excretion route may contribute to this phenomenon. However, further studies are required to prove this hypothesis as there were no dose-dependent differences in drug clearance in the MAD study. Thus, the dose proportionality of the PK parameters of izuforant could not be defined by considering the different results of the SAD and MAD studies. Therefore, further clinical studies are required to prove the dose proportionality of izuforant.

A decrease in izuforant clearance was observed with dose escalation in the SAD study, leading to a more than proportional increase in exposure, a trend not evident in the MAD study. Non-clinical studies identified izuforant as a P-glycoprotein (P-gp) substrate and an inhibitor of MATE1, MATE2-K, and OCT2, which are involved in renal active secretion (unpublished data). The saturable nature of these transporters may explain the dose-dependent decrease in izuforant clearance.

When only the PK profile was considered, a period shorter than 7 days could suffice in a steady state based on the $t_{1/2}$ of izuforant. However, a 7-day once-daily dose regimen was selected to obtain sufficient information about the PD profile, considering the situation where the drug is administered to a patient in a clinical setting.

Histamine-mediated ESC have been considered a validated biomarker for evaluating H4R antagonism and have been used to assess the PD effect of H4R antagonists in both non-clinical and clinical studies.[28–31] In addition to histamine, Imetit, an H4R agonist, has been reported to change the shape of eosinophils in an in vitro study.[13,32] In the validation step for setting the PD assay protocol, imetit was considered more capable of inducing ESC in a dose-dependent manner and causing maximal change in eosinophil count. Therefore, imetit was used to induce shape changes in eosinophils in this clinical study.

A repeated mixed model analysis was conducted for the statistical comparison of imetit-induced ESC in this study, unlike the one-way ANOVA used in clinical studies of other H4R antagonists.[30,31] Although there is no established rule for selecting an appropriate method for statistical analysis, it was concluded that the repeated mixed model analysis was more appropriate for considering the properties of the variable since one-way ANOVA is more useful for considering the individual variability of the baseline eosinophil FSC and the corresponding imetit-induced ESC.

The mean number of imetit-induced ESC at baseline in the SAD study tended to be higher than that in the MAD study. The observed FSC_i tended to be smaller in the MAD study than in the SAD study, while the observed FSC_b tended to be larger in the MAD study than in the SAD study. We speculate that the time difference between the schedules of the SAD and MAD studies may have affected the mean imetit-induced ESC. Both inter- and intra-individual variability in the study subjects, reflected in the mean eosinophil FSC, could have also affected imetit-induced ESC. Therefore, further studies are needed to reveal the primary factors that affect imetit-induced ESC.

The imetit-induced ESC in the 30 mg dose group in the SAD study did not respond to immediate stimulation, as observed throughout the study period. The use of an inappropriate erythrocyte lysis buffer during the assay was concluded to be the cause of inconsistent data because the phenomenon did not occur after using another batch of erythrocyte lysis buffer.

The dose–response relationship was examined after multiple administrations of izuforant. When 400 mg of izuforant was administered, the PD parameter $AUEC_{ss}$, which reflects the extent and duration of the ESC

TABLE 3 Summary of treatment emergent adverse events by system organ class and preferred term (SAD study).

System organ class preferred term	No. of subjects (percentage of subjects with adverse events) [No. of adverse events]						
	Placebo ($n=10$)	10 mg ($n=6$)	30 mg ($n=6$)	100 mg ($n=6$)	300 mg ($n=6$)	600 mg ($n=6$)	Total ($n=40$)
Subjects with any adverse event	2 (20.0) [4]	2 (33.3) [2]	1 (16.7) [2]	1 (16.7) [1]	1 (16.7) [1]	6 (100.0) [8]	13 (32.5) [18]
Nervous system disorders	1 (10.0) [1]	–	–	1 (16.7) [1]	–	4 (66.7) [4]	6 (15.0) [6]
Dizziness	–	–	–	–	–	3 (50.0) [3]	3 (7.5) [3]
Headache	1 (10.0) [1]	–	–	1 (16.7) [1]	–	–	2 (5.0) [2]
Syncope	–	–	–	–	–	1 (16.7) [1]	1 (2.5) [2]
Gastrointestinal disorders	–	1 (16.7) [1]	–	–	–	2 (33.3) [2]	3 (7.5) [3]
Abdominal pain	–	–	–	–	–	1 (16.7) [1]	1 (2.5) [1]
Gastrointestinal hemorrhage	–	1 (16.7) [1]	–	–	–	–	1 (2.5) [1]
Nausea	–	–	–	–	–	1 (16.7) [1]	1 (2.5) [1]
Investigations	1 (10.0) [2]	–	1 (16.7) [2]	–	1 (16.7) [1]	–	3 (7.5) [5]
White blood cell count decreased	1 (10.0) [1]	–	–	–	1 (16.7) [1]	–	2 (5.0) [2]
Aspartate aminotransferase increased	–	–	1 (16.7) [1]	–	–	–	1 (2.5) [1]
Blood creatine phosphokinase increased	–	–	1 (16.7) [1]	–	–	–	1 (2.5) [1]
Neutrophil count decreased	1 (10.0) [1]	–	–	–	–	–	1 (2.5) [1]
Infections and infestations	1 (10.0) [1]	–	–	–	–	1 (16.7) [1]	2 (5.0) [2]
Hordeolum	–	–	–	–	–	1 (16.7)/ [1]	1 (2.5) [1]
Pharyngitis	1 (10.0) [1]	–	–	–	–	–	1 (2.5) [1]
Metabolism and nutrition disorders	–	–	–	–	–	1 (16.7)/ [1]	1 (2.5) [1]
Hypertriglyceridemia	–	–	–	–	–	1 (16.7)/ [1]	1 (2.5) [1]
Respiratory, thoracic and mediastinal disorders	–	1 (16.7) [1]	–	–	–	–	1 (2.5) [1]
Epistaxis	–	1 (16.7) [1]	–	–	–	–	1 (2.5) [1]

Abbreviation: SAD, single ascending dose.

inhibition effect, was only ~13% higher than when 200 mg izuforant was administered, despite doubling the dose. Therefore, the increase in efficacy following administering a 400 mg dose is not of interest given the safety aspects such as the number of AE incidents and the number of subjects who experienced AEs.

In vitro studies have shown that izuforant is a substrate of P-glycoprotein (P-gp) (unpublished data). Therefore, the likelihood of the drug crossing the blood–brain barrier is low, suggesting that some AE observed in this study, such as nausea, vomiting, and dizziness, are unlikely to originate from the central nervous system. Other H4R antagonists are under clinical development, and some H4R antagonists have shown favorable efficacy in clinical studies.[33,34] One tolerability issue observed with izuforant dosing was a dose-dependent increase in gastrointestinal AEs similar to that observed with other H4R antagonists[30,31]; this issue might be due to a local nonspecific effect in the stomach and might be improved by modifying the formulation of the IPs, as in other clinical studies on H4R antagonists.[31] However, further clinical research is required to confirm these hypotheses. Clinical studies are also required to prove the safety of izuforant in patients with AD, considering agranulocytosis, which was reported in a previous H4R antagonist clinical study.[35]

In a phase II clinical trial evaluating the efficacy and safety of izuforant in patients with cholinergic urticaria, a dosing regimen of 100 mg twice daily was used.[36] The total daily dose of 200 mg was selected as the intermediate dose

TABLE 4 Summary of treatment emergent adverse events by system organ class and preferred term (MAD study).

System organ class preferred term	No. of subjects (percentage of subjects with adverse events) [No. of adverse events]				
	Placebo ($n=6$)	100 mg ($n=6$)	200 mg ($n=6$)	400 mg ($n=6$)	Total ($n=24$)
Subjects with any adverse event	1 (16.7) [1]	1 (16.7) [1]	3 (50.0) [3]	5 (83.3) [30]	10 (41.7) [35]
Nervous system disorders	–	–	1 (16.7) [1]	3 (50.0) [5]	4 (16.7) [6]
Dizziness	–	–	–	3 (50.0) [5]	3 (12.5) [5]
Headache	–	–	1 (16.7) [1]	–	1 (4.2) [1]
Gastrointestinal disorders	–	–	–	4 (66.7) [23]	4 (16.7) [23]
Diarrhea	–	–	–	2 (33.3) [2]	2 (8.3) [2]
Abdominal distension	–	–	–	2 (33.3) [3]	2 (8.3) [3]
Abdominal pain	–	–	–	3 (50.0) [6]	3 (12.5) [6]
Nausea	–	–	–	3 (50.0) [10]	3 (12.5) [10]
Vomiting	–	–	–	1 (16.7) [2]	1 (4.2) [2]
Investigations	1 (16.7) [1]	–	–	1 (16.7) [1]	2 (8.3) [2]
Blood creatine phosphokinase increased	–	–	–	1 (16.7) [1]	1 (4.2) [1]
Blood pressure increased	1 (16.7) [1]	–	–	–	1 (4.2) [1]
Blood and lymphatic system disorders	–	1 (16.7) [1]	–	1 (16.7) [1]	2 (8.3) [2]
Anemia	–	1 (16.7) [1]	–	1 (16.7) [1]	2 (8.3) [2]
Metabolism and nutrition disorders	–	–	1 (16.7) [1]	–	1 (4.2) [1]
Hypertriglyceridemia	–	–	1 (16.7) [1]	–	1 (4.2) [1]
Respiratory, thoracic, and mediastinal disorders	–	–	1 (16.7) [1]	–	1 (4.2) [1]
Epistaxis	–	–	1 (16.7) [1]	–	1 (4.2) [1]

Abbreviation: MAD, multiple ascending dose.

among the dose groups in this study's MAD study, chosen based on the efficacious dose from ESC assay. Based on the pharmacodynamic evaluation results of this clinical trial, a once-daily dosing regimen of izuforant could be considered. However, given the reported half-life of ~7 h, it is presumed that a twice-daily regimen was used in the phase II clinical trial to ensure consistent exposure throughout the day.

The present study has some limitations. Imetit-induced ESCs were used as PD end points in the study. Although this reflects the mechanism of allergic reactions via the H4R pathway at the cellular level, ESCs do not show a strong association with clinical symptoms of AD. Thus, the development of surrogate biomarkers that can quantifiably link clinical symptoms such as pruritus and inflammation with allergic reactions that occur via the H4R pathway is desirable.

AUTHOR CONTRIBUTIONS

B.H.J, T.H, and M.S.P wrote the manuscript; B.H.J, T.H, B.W.Y, C.O.K, D.K, and M.S.P designed the research; B.H.J, T.H, B.W.Y, C.O.K, D.K, and M.S.P performed the research; and T.H, B.H.J, D.K, Y.N.K, and M.S.P analyzed the data.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

1. Thangam EB, Jemima EA, Singh H, et al. The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: the hunt for new therapeutic targets. *Front Immunol*. 2018;9:1873.
2. Breunig E, Michel K, Zeller F, Seidl S, Weyhern CW, Schemann M. Histamine excites neurones in the human submucous plexus through activation of H1, H2, H3 and H4 receptors. *J Physiol*. 2007;583(Pt 2):731-742.
3. Connelly WM, Shenton FC, Lethbridge N, et al. The histamine H4 receptor is functionally expressed on neurons in the mammalian CNS. *Br J Pharmacol*. 2009;157(1):55-63.
4. Horr B, Borck H, Thurmond R, Grosch S, Diel F. STAT1 phosphorylation and cleavage is regulated by the histamine (H4) receptor in human atopic and non-atopic lymphocytes. *Int Immunopharmacol*. 2006;6(10):1577-1585.
5. Liu C, Ma X, Jiang X, et al. Cloning and pharmacological characterization of a fourth histamine receptor (H(4)) expressed in bone marrow. *Mol Pharmacol*. 2001;59(3):420-426.
6. Medina VA, Rivera ES. Histamine receptors and cancer pharmacology. *Br J Pharmacol*. 2010;161(4):755-767.
7. Morgan RK, McAllister B, Cross L, et al. Histamine 4 receptor activation induces recruitment of FoxP3+ T cells and inhibits allergic asthma in a murine model. *J Immunol*. 2007;178(12):8081-8089.
8. Nakamura T, Itadani H, Hidaka Y, Ohta M, Tanaka K. Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem Biophys Res Commun*. 2000;279(2):615-620.
9. Oda T, Morikawa N, Saito Y, Masuho Y, Matsumoto S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem*. 2000;275(47):36781-36786.
10. Yamaura K, Shigemori A, Suwa E, Ueno K. Expression of the histamine H4 receptor in dermal and articular tissues. *Life Sci*. 2013;92(2):108-113.
11. Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J Pharmacol Exp Ther*. 2003;305(3):1212-1221.
12. Lim HD, van Rijn RM, Ling P, Bakker RA, Thurmond RL, Leurs R. Evaluation of histamine H1-, H2-, and H3-receptor ligands at the human histamine H4 receptor: identification of 4-methylhistamine as the first potent and selective H4 receptor agonist. *J Pharmacol Exp Ther*. 2005;314(3):1310-1321.
13. Ling P, Ngo K, Nguyen S, et al. Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br J Pharmacol*. 2004;142(1):161-171.
14. Winder SJ, Ayscough KR. Actin-binding proteins. *J Cell Sci*. 2005;118(Pt 4):651-654.
15. Bautista DM, Wilson SR, Hoon MA. Why we scratch an itch: the molecules, cells and circuits of itch. *Nat Neurosci*. 2014;17(2):175-182.
16. Ikoma A, Steinhoff M, Ständer S, Yosipovitch G, Schmelz M. The neurobiology of itch. *Nat Rev Neurosci*. 2006;7(7):535-547.
17. Buddenkotte J, Steinhoff M. Pathophysiology and therapy of pruritus in allergic and atopic diseases. *Allergy*. 2010;65(7):805-821.
18. Kim K. Neuroimmunological mechanism of pruritus in atopic dermatitis focused on the role of serotonin. *Biomol Ther (Seoul)*. 2012;20(6):506-512.
19. Nakano T, Andoh T, Lee JB, Kuraishi Y. Different dorsal horn neurons responding to histamine and allergic itch stimuli. *Neuroreport*. 2008;19(7):723-726.
20. Abramovits W. Atopic dermatitis. *J Am Acad Dermatol*. 2005;53(1 Suppl 1):S86-S93.
21. Leung DY, Bieber T. Atopic dermatitis. *Lancet*. 2003;361(9352):151-160.
22. McPherson T. Current understanding in pathogenesis of atopic dermatitis. *Indian J Dermatol*. 2016;61(6):649-655.
23. Denby KS, Beck LA. Update on systemic therapies for atopic dermatitis. *Curr Opin Allergy Clin Immunol*. 2012;12(4):421-426.
24. Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis–part II: immune cell subsets and therapeutic concepts. *J Allergy Clin Immunol*. 2011;127(6):1420-1432.
25. Novak N, Simon D. Atopic dermatitis - from new pathophysiologic insights to individualized therapy. *Allergy*. 2011;66(7):830-839.
26. FDA US. *Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers*. U.S. Food and Drug Administration; 2005.
27. Smith BP, Vandenhende FR, DeSante KA, et al. Confidence interval criteria for assessment of dose proportionality. *Pharm Res*. 2000;17(10):1278-1283.
28. Barnard R, Barnard A, Salmon G, Liu W, Sreckovic S. Histamine-induced actin polymerization in human eosinophils: an imaging approach for histamine H4 receptor. *Cytometry A*. 2008;73(4):299-304.
29. Mowbray CE, Bell AS, Clarke NP, et al. 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*. 2011;21(21):6596-6602.
30. Thurmond RL, Chen B, Dunford PJ, et al. Pharmacology and clinical activity of toreforant, a histamine H4 receptor antagonist. *Ann Pharmacol Pharmaceut*. 2017;2(1):1013.
31. Thurmond RL, Chen B, Dunford PJ, et al. Clinical and preclinical characterization of the histamine H(4) receptor antagonist JNJ-39758979. *J Pharmacol Exp Ther*. 2014;349(2):176-184.
32. Buckland KF, Williams TJ, Conroy DM. Histamine induces cytoskeletal changes in human eosinophils via the H(4) receptor. *Br J Pharmacol*. 2003;140(6):1117-1127.
33. Boyle DL, DePrimo SE, Calderon C, et al. Toreforant, an orally active histamine H(4)-receptor antagonist, in patients with active rheumatoid arthritis despite methotrexate: mechanism of action results from a phase 2, multicenter, randomized, double-blind, placebo-controlled synovial biopsy study. *Inflamm Res*. 2019;68(4):261-274.
34. Werfel T, Layton G, Yeadon M, et al. Efficacy and safety of the histamine H(4) receptor antagonist ZPL-3893787 in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2019;143(5):1830-1837.e1834.
35. Murata Y, Song M, Kikuchi H, et al. Phase 2a, randomized, double-blind, placebo-controlled, multicenter, parallel-group study of a H4 R-antagonist (JNJ-39758979) in

Japanese adults with moderate atopic dermatitis. *J Dermatol*. 2015;42(2):129-139.
36. Grekowitz E, Metz M, Altrichter S, et al. Targeting histamine receptor 4 in cholinergic urticaria with izuforant (LEO 152020): results from a phase IIa randomized double-blind placebo-controlled multicentre crossover trial. *Br J Dermatol*. 2024;190(6):825-835.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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