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RESEARCH ARTICLE

Development of a pharmacokinetic/pharmacodynamic/disease progression model in NC/Nga mice for development of novel anti-atopic dermatitis drugs

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Abstract

1. JHL45, a novel immune modulator against atopic dermatitis (AD), was synthesized from decursin isolated from *Angelica gigas*. The goal is to evaluate the lead compound using quantitative modeling approaches to novel anti-AD drug development.
2. We tested the anti-inflammatory effect of JHL45 by *in vitro* screening, characterized its *in vitro* pharmacokinetic (PK) properties. The dose-dependent efficacy of JHL45 was developed using a pharmacokinetics/pharmacodynamics/disease progression (PK/PD/DIS) model in NC/Nga mice.
3. JHL45 has drug-like properties and pharmacological effects when administered orally to treat atopic dermatitis. The developed PK/PD/DIS model described well the rapid metabolism of JHL45, double-peak phenomenon in the PK of decursinol and inhibition of IgE generation by compounds in NC/Nga mice. Also, a quantitative model was developed and used to elucidate the complex interactions between serum IgE concentration and atopic dermatitis symptoms.
4. Our findings indicate that JHL45 has good physicochemical properties and powerful pharmacological effects when administered orally for treatment of AD in rodents.

Keywords

Atopic dermatitis, disease progression model, drug development, pharmacodynamics, pharmacokinetics

History

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Introduction

Atopy is defined as familial hypersensitivity of the skin and the mucosa to environmental substances, associated with an increased production of immunoglobulin E (IgE) and/or altered pharmacological reactivity (Novak, 2009). Atopic dermatitis (AD), one of the most common skin diseases in children with a family history of atopy, is an eczematous disorder of the skin that affects up to 20% of children and 1–3% of adults (Strachan, 1999). The pathophysiology of AD is complex and triggering factors vary among patients. However, rapidly increasing knowledge of the complex background at the genetic (Holloway et al., 2010), immunological (Yamanaka & Mizutani, 2011) and environmental (Arshad, 2005; Plötz & Ring, 2010) levels has improved the diagnostic options for AD and suggested new treatment approaches (Buddenkotte & Steinhoff, 2010; Novak & Simon, 2011; Ständer & Luger, 2010).

Presently available treatments for AD include antihistamines, emollients, doxepin and corticosteroids

(Furie et al., 2011). Topical steroids are commonly used to treat AD, but their chronic use at high concentrations can cause severe adverse effects such as skin atrophy, pigmentation alterations, hypothalamic changes and growth inhibition (Blume-Peytavi & Wahn, 2011; Svensson et al., 2011). Accordingly, the safety and pathophysiological characteristics of new AD therapies should be evaluated as soon as possible. In this context, recent research indicates that natural medicines and derivatives have scientific merit and clinical benefits (Dunstan et al., 2011; Lee & Bielory, 2010).

Decursin is a pyranocoumarin found in the roots of *Angelica gigas* Nakai, which has been traditionally used to treat anemia and to benefit women's health, and as a sedative or an anodyne agent (Jung et al., 1991). It has been reported that decursin has anti-tumor, anti-bacterial, anti-inflammatory, anti-oxidant and cognitive-enhancing activities and produces improvements in the circulatory system (Son et al., 2009). We hypothesized that the anti-inflammatory activity of decursin is associated with the suppression of cytokine and immunoglobulin E (IgE) release and that it would be effective against AD.

3-(3,4-Dihydroxy-phenyl)-acrylic acid 2,2-dimethyl-8-oxo-3,4-dihydro-2*H*,8*H*-pyrano[3,2-*g*]chromen-3-yl-ester (JHL45) is a decursin derivative and a new agent with anti-allergic and anti-inflammatory effects. Its pharmacological properties are superior to those of decursin

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(Yang et al., 2009). JHL45 is rapidly metabolized to decursinol after intravenous administration in rats (Baek et al., 2009). Decursinol has similar pharmacological effects to decursin, exhibits high permeability, has high stability in liver microsomes and has dose-dependent pharmacokinetics (PK) (Song et al., 2011).

Animal model has been considered to play an important role in the characterization of disease pathophysiology, target validation and *in vivo* evaluation of novel drug development. Especially, disease animal models have been utilized in describing the relationships between pharmacokinetics (PK) and pharmacodynamics (PD) (McGonigle & Ruggeri, 2014). NC/Nga mice are most appropriate AD animal model that reflects the human AD with IgE hyperproduction (Matsuda et al., 1997). NC/Nga mice spontaneously develop atopic dermatitis-like skin lesion such as erythema, edema, bleeding, hair loss, skin dryness and scratching behaviors similar to those seen in human AD patients (Suto et al., 1999).

In the present study, we tested the anti-inflammatory effect of JHL45 by *in vitro* screening, characterized its *in vitro* PK properties (solubility, permeability, metabolic stability, plasma-protein binding), and subjected it to a CYP450 assay. We also investigated the PK of JHL45 following intravenous and oral administration to rats, developed a pharmacokinetics/pharmacodynamics/disease progression (PK/PD/DIS) model to identify potential surrogates, and characterized the dose-dependent efficacy of JHL45 in NC/Nga mice. The ultimate objective of this study was to evaluate the lead compound using quantitative modeling approaches to novel anti-AD drug development.

Materials and methods

Chemicals and reagents

JHL45 (purity >98%) was synthesized from decursin as described previously (Yang et al., 2009). Decursinol, the metabolite of JHL45, was extracted from the roots of *Angelica gigas* (Lee et al., 2006). Verapamil and 7-hydroxycoumarin were purchased from Sigma Chemical Co. (St. Louis, MO). Pooled human and rat liver microsomes, an NADPH-regenerating system and UGT reaction mix solution were obtained from BD Gentest (Woburn, MA). HPLC-grade organic solvents were purchased from Merck Co. (Darmstadt, Germany). All chemicals and solvents were of the highest analytical grade available.

In vitro screening

Human monocytic THP-1 cells and the human eosinophilic leukemia EoL-1 cells were obtained from the American Type Culture Collection (Manassas, VA) and Riken Cell Bank (Tsukuba, Japan), respectively, and were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 µg/mL).

THP-1 cells and EoL-1 cells were treated with house dust-mite (HDM) extract after pretreatment with JHL45 for 30 min. Concentrations of interleukin-6 (IL-6), IL-8 and monocyte chemoattractant protein-1 (MCP-1) in supernatants were measured by the sandwich enzyme-linked

immunosorbent assay (ELISA) using an OptEIA Set (BD Biosciences, San Diego, CA), according to the manufacturer's instructions. All assays were performed in triplicate. Cytokine concentrations were calculated using a linear-regression equation obtained from the absorbance values of standards.

Solubility

To determine the kinetic solubility of JHL45 and decursinol, stock solutions (10 mmol/L) were manually prepared in 5% DMSO: 95% phosphate-buffered saline (PBS) buffer. For a single batch of compounds, the stock solutions were diluted serially in wells across the plates with PBS buffer containing 5% DMSO. Each plate was read vertically, with a gain of 30 and a laser intensity of 90%, to produce raw data (counts per well). All raw data were processed using BMG Labtech NEPHELOstar Galaxy evaluation software (Offenburg, Germany). The intrinsic equilibrium solubility of compounds was measured by the chasing equilibrium solubility (CheqSol) method (Stuart & Box, 2005).

Permeability

The permeability of JHL45 and decursinol was determined by Parallel Artificial Membrane Permeability Assay (PAMPATM, Millipore, Billerica, MA) (Kansy et al., 1998). Briefly, 1% (w/v) lecithin in dodecane was prepared by sonication and 5 µL of the lecithin/dodecane solution was pipetted into each donor plate well of a drug-filtration plate, ensuring that the pipette tip did not make contact with the membrane. Immediately after the application of lecithin, 150 µL of compound dissolved in PBS containing 5% DMSO was injected into each well of the donor plate. Approximately 300 µL of buffer containing 5% DMSO was then added to each well of the acceptor plate. The donor plate was gently placed into the acceptor plate, making sure that the underside of the membrane was in contact with the buffer in all wells. The plate lid was replaced and the plate was incubated, at room temperature in a sealed container, for 16 h. After incubation, the UV/Vis absorption from 250 to 500 nm of the donor solution (100 µL/well) and acceptor solution (250 µL/well) was measured. The apparent permeability coefficient (P_{app}) for each test compound was calculated using the following equation:

$$P_{app} = C \times \left(-\ln \left(1 - \frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{equilibrium}}} \right) \right)$$

where

$$C = \frac{\text{Volume Donor} \times \text{Volume Acceptor}}{\left\{ (\text{Volume Donor} + \text{Volume Acceptor}) \times \text{Peak area} \times \text{Time} \right\}}$$

Partition coefficient *P* (log *P*) and ionization (p*K*_a)

Partition coefficient *P* (log *P*) and p*K*_a were determined using a GLp*K*_a automatic titrator (Sirius, Forest Row, UK). To determine the log *P* values of JHL45 and decursinol, the value of *r* (*n*-octanol/0.15 M KCL) was between 0.0025 and 18, the aqueous volume was ≥5 mL, and *n*-octanol was saturated

with 0.15 M KCl prior to dosing. To determine pK_a , titration of 50 μ M JHL45 and decursinol in 20 mL of 0.15 M KCl was performed using a Sirius analytical instrument. Finally, log P and pK_a values were obtained using the pK_a LOGPTM software [version 5.01, Sirius (Avdeef, 1993)].

Metabolic stability assay

The metabolic stability of JHL45 and decursinol was determined in rat and human liver microsomes. Liver microsomes (final protein concentration 0.5 mg/mL) were suspended in 100 mM potassium phosphate buffer (pH 7.4) and compounds were tested at a concentration of 10 μ M. The reaction was started by the addition of NADPH-regenerating solution, which contains 1.3 mM NADP⁺, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM MgCl₂. Single sample was collected at 30 min. The reaction was terminated by the addition of an equal volume of cold acetonitrile. Samples were centrifuged at 4 °C for 5 min at 13 000 rpm. The clear supernatant was collected and analyzed using an API-2000 LC-MS/MS system (Applied Biosystems, Foster City, CA).

Plasma-protein binding

The fraction of JHL45 and decursinol not bound to rat plasma protein was estimated using an ultrafiltration method. Human plasma samples were prepared with test compounds (2 μ g/mL) in triplicate. After incubation at 37 °C for 30 min, a 0.5-mL aliquot was transferred to an Amicon Centrifree Micropartition device (30 000-Da cut-off YMT membrane; Millipore, Bedford, MA). After centrifugation at 2000 $\times g$ for 20 min at 37 °C, filtrates were analyzed using the API-2000 LC-MS/MS system (Applied Biosystems). Percentage protein binding was calculated using the following formula:

$$\text{Plasma protein binding} = \left(1 - \frac{[\text{drug}]_{\text{ultrafiltrate}}}{[\text{drug}]_{\text{total}}} \right) \times 100$$

Cytochrome P450 inhibition assay

The potential of JHL45 and decursinol to inhibit major human CYP enzymes was evaluated using a Vivid[®] CYP450 screening kit protocol (PanVera; Invitrogen, Carlsbad, CA). Briefly, 40 μ L of test compound solutions of various concentrations and 50 μ L of a mixture of CYP450 BACULOSOME[®] Reagents and Regeneration System in Vivid[®] CYP450 Reaction Buffer were added to a 96-well plate. The mixtures were pre-incubated for 20 min at room temperature. The reaction was then initiated by the addition of 10 μ L of a mixture of reconstituted substrate and NADP⁺ in Vivid[®] CYP450 Reaction Buffer. Fluorescence of the CYP isoforms was measured using a SpectraMax plate reader (Molecular Devices, Silicon Valley, CA) with an excitation wavelength of 405 nm and an emission wavelength of 460 nm. Probe substrates were phenacetin, midazolam, diclofenac, S-mephenytoin and dextromethorphan for CYP1A2, 3A4, 2C9, 2C19 and 2D6, respectively. α -Naphthoflavone, ketoconazole, sulfaphenazole, tranlycypromine and quinidin were identified as positive control inhibitors for CYP1A2, 3A4, 2C9, 2C19 and 2D6, respectively.

LC-MS/MS assay

The plasma concentrations of JHL45 and decursinol were measured simultaneously by LC-MS/MS as previously reported (Baek et al., 2009). Briefly, the mass transitions m/z 408.8 $\rightarrow$ 229.0 for JHL45, m/z 246.8 $\rightarrow$ 90.4 for decursinol, and m/z 152.0 $\rightarrow$ 109.8 for an internal standard (IS, acetaminophen) were monitored in multiple reaction monitoring (MRM) mode. JHL45, decursinol and the IS were extracted from the plasma matrix by protein precipitation. Samples were injected to a reverse-phase column (Xterra[®] C₁₈, 2.1 $\times$ 50-mm i.d. 3.5- μ m particle size; Waters, Milford, MA) with a mobile phase of 0.1% formic acid in a mixture of methanol and purified water (98:2, v/v). Linearity was achieved within the limits of quantitation (10 ng/mL for JHL45, 50 ng/mL for decursinol). The intra- and inter-day precision of the assay ranged from 0.74 to 13.16%, and the intra- and inter-day accuracy from 90.06 to 108.27%. Short-term, long-term, freeze-thaw and extraction stabilities were acceptable.

Pharmacokinetics of JHL45 and its metabolite in rats after intravenous and oral administration

All animal experiments were carried out in accordance with the standard operating procedures of our institute. The study was approved by the Institutional Review Board of Chungnam National University. Twenty male SD rats ($n = 5$ per group) had polyethylene tubing (PE-50; Intramedic, Franklin Lakes, NJ) implanted into the jugular and femoral veins under ketamine-induced anesthesia and were fasted for 12 h before drug dosing. The cannula was filled with normal saline containing heparin (20 IU/mL) to prevent clotting. The vehicle used for intravenous (1 mg/kg, 5 mg/kg) and oral (1 mg/kg, 5 mg/kg) dosing was dimethyl sulfoxide/cremophore/polyethylene glycol 400/distilled water (5:5:60:30%). Blood samples were collected from the femoral vein of each rat before administration and at 0.03 (IV), 0.08 (IV), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after drug administration. The plasma was separated from 50- μ L blood samples by centrifugation and stored at -70 °C until LC-MS/MS analysis.

Pharmacokinetic analyses were performed by the non-compartmental method with WinNonlin Standard Edition software, version 4.1 (Pharsight Corp., Mountain View, CA). The area under the plasma concentration-versus-time curve from time zero to the last measurable concentration (AUC) was calculated using the linear trapezoidal rule and was extrapolated to infinity (AUC_{inf}). The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) of JHL45 and decursinol in the plasma were obtained directly from the data. An elimination rate constant (k_{el}) was calculated by log-linear regression of the terminal phase, and the elimination half-life ($t_{1/2}$) was calculated as $0.693/k_{\text{el}}$. Total body clearance (CL) and steady-state volume of distribution (V_{ss}) were calculated as $\text{Dose}/AUC_{\text{inf}}$ and $CL \cdot MRT$. The results are presented as means $\pm$ standard deviations.

Pharmacokinetics, pharmacodynamics and skin severity score in NC/Nga mice

Five-week-old male NC/Nga mice (SLC Japan Inc., Shizuoka, Japan) were housed in an animal facility at the College of

Pharmacy, Chungnam National University. All animal experiments were carried out in accordance with the standard operating procedures of the facility. The study was approved by the Institutional Review Board of Chungnam National University. AD was induced in NC/Nga mice using 2,4-dinitrochlorobenzene (DNCB), as described previously (Lee et al., 2011). Briefly, the dorsal hair of NC/Nga mice was shaved-off using an electric shaver, and 1% DNCB solution in a 3:1 acetone:olive oil mixture was applied to the shaved dorsal skin. After this initial sensitization, the mice were dorsally treated with 0.3% DNCB at 1-week intervals for 4 weeks.

The skin severity score was used to evaluate treatment with JHL45 (1, 3 and 10 mg/kg) ($n = 10$ per group). The extent of (1) pruritus/itching, (2) erythema/hemorrhage, (3) edema, (4) excoriation/erosion and (5) scaling/dryness was scored as 0 (no symptom), 1 (mild), 2 (moderate) and 3 (severe) (Suto et al., 1999). The total skin severity score was defined as the sum of the individual scores. The vehicle for oral JHL45 (1, 3 and 10 mg/kg) was PBS buffer. JHL45 was administered to the NC/Nga mice orally once per day for 3 weeks using an oral zoned needle. Blood samples (<0.3 mL) were collected from the ocular plexus venous of each rat at 0, 0.05, 1, 2 and 8 h after dosing on days 1, 7, 14 for PK and PD analysis. Plasma concentrations of JHL45 and decursinol were measured by LC-MS/MS, and total serum IgE concentrations were measured using a sandwich ELISA kit (R&D Systems Inc. Minneapolis, MN), according to the manufacturer's instructions. Skin severity score was assessed macroscopically every week in a blinded fashion during the 3-week treatment period.

Mice were sacrificed at 3 weeks after JHL45 treatment. Single-cell suspensions were prepared from the spleen and incubated at 37 °C for 24 h. Culture supernatants were then collected. The concentrations of IL-4, IL-5, IL-6 and MCP-1 were determined by the ELISA using an OptEIA Set

(BD Biosciences, San Diego, CA), and concentrations of IL-13 were measured using a DuoSet ELISA kit (R&D Systems) (Lee et al., 2011).

Pharmacokinetic/pharmacodynamic/disease progression model in NC/Nga mice

An integrated mechanism-based PK/PD/DIS model was proposed and is shown in Figure 1. The model reflects the double-peak phenomenon in the PK of decursinol, the inhibitory effect on serum IgE of an indirect PD response (Krzyzanski & Jusko, 1998), and improvement in skin severity by symptomatic drug effect (Chan & Holford, 2011). The time-concentration profiles of JHL45 and decursinol were modeled in advance, and PD and disease progression were subsequently modeled with fixed PK parameters.

The plasma concentration-versus-time profiles of JHL45 and decursinol were fitted to an enterohepatic circulation (EHC) model. Previous reports that decursinol undergoes non-renal clearance, producing a second peak in the oral response, but no second peak in the intravenous response, supports the EHC model (Godfrey et al., 2011; Song et al., 2011). Equations (1)–(8) describe the changes in the amounts of JHL45 and decursinol in the compartments following oral administration.

$$\frac{dA_{\text{gut}}}{dt} = -k_a \cdot A_{\text{gut}} - k_{\text{am}} \cdot A_{\text{gut}} \quad (1)$$

$$\frac{dA_{\text{jhl}}}{dt} = k_a \cdot A_{\text{gut}} - (k_{\text{el}} + k_{\text{m}}) \cdot A_{\text{jhl}} \quad (2)$$

$$\frac{dA_{\text{decur}}}{dt} = k_{\text{am}} \cdot A_{\text{gut}} + k_{\text{m}} \cdot A_{\text{jhl}} - (k_{\text{elm}} + k_{\text{mr}}) \cdot A_{\text{decur}} \quad (3)$$

$$\frac{dA_{\text{r}}}{dt} = k_{\text{mr}} \cdot A_{\text{decur}} \quad (4)$$

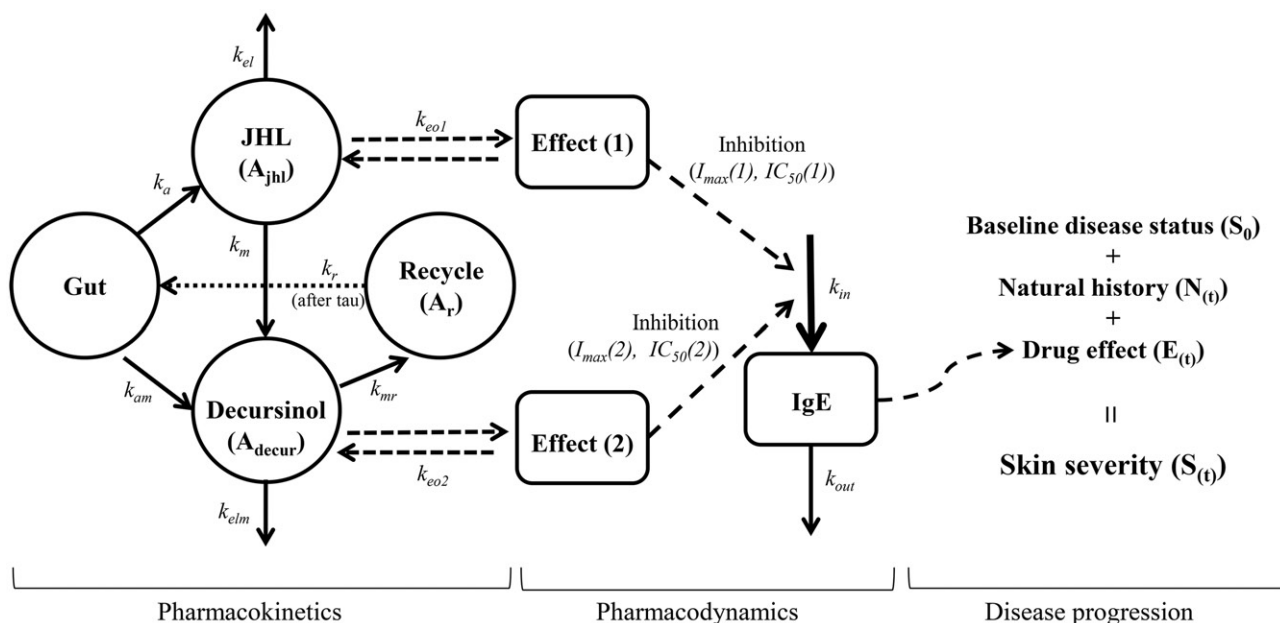


Figure 1. PK/PD/DIS model of JHL45.

If time is greater than recycle time (τ), then:

$$\frac{dA_{\text{gut}}}{dt} = -k_{\text{am}} \cdot A_{\text{gut}} + k_{\text{r}} \cdot A_{\text{r}} \quad (5)$$

$$\frac{dA_{\text{jhl}}}{dt} = -(k_{\text{el}} + k_{\text{m}})A_{\text{jhl}} \quad (6)$$

$$\frac{dA_{\text{decur}}}{dt} = k_{\text{am}} \cdot A_{\text{gut}} + k_{\text{m}} \cdot A_{\text{jhl}} - (k_{\text{elm}} + k_{\text{mr}}) \cdot A_{\text{decur}} \quad (7)$$

$$\frac{dA_{\text{r}}}{dt} = k_{\text{mr}} \cdot A_{\text{decur}} - k_{\text{r}} \cdot A_{\text{r}} \quad (8)$$

where A_{gut} and A_{jhl} represent the amounts of JHL45 in the gut and central compartments; A_{decur} and A_{r} are the amounts of decursinol in the metabolite and recycling compartments; k_{a} and k_{am} are the first-order absorption rate constants for JHL45 and decursinol; k_{el} and k_{elm} are the first-order elimination rate constants for JHL45 and decursinol; k_{m} is the formation rate constant of the drug from the JHL45 compartment to the metabolite compartment; k_{mr} is the first-order rate constants for JHL45 and decursinol excreted into bile (the recycling compartment); and k_{r} is the first-order rate constant for the dispersion of decursinol into the gut compartment at the time of recycling.

Equations (1–8) were solved numerically and fitted to the data using ADAPT 5 (Biomedical Simulation Resource, Los Angeles, CA) with a maximum-likelihood estimation under the assumption that the standard deviation of the measurement error was a linear function of the measured quantity. Plasma concentrations of JHL45 (C_{pjl}) and decursinol (C_{pdecur}) were calculated using the following equations:

$$C_{\text{pjl}} = A_{\text{jhl}}/V_{\text{jhl}} \quad (9)$$

$$C_{\text{pdecur}} = A_{\text{decur}}/V_{\text{decur}} \quad (10)$$

where V_{jhl} and V_{decur} are the volumes of distribution of JHL45 and decursinol. The final model accommodated fast elimination of JHL45 and the secondary peaks of decursinol, with elimination into a recycling compartment and instantaneous release back into the gut after a fitted recycling time.

Inhibitory effects on serum IgE were fitted using an indirect response model with production of the response variable inhibited by the concentration of JHL45 and decursinol in an effect site (Sheiner et al., 1979):

$$\frac{dE_{\text{jhl}}}{dt} = k_{\text{eo1}} \cdot \left(\frac{A_{\text{jhl}}}{V_{\text{jhl}}} - E_{\text{jhl}} \right) \quad (11)$$

$$\frac{dE_{\text{decur}}}{dt} = k_{\text{eo2}} \cdot \left(\frac{A_{\text{decur}}}{V_{\text{decur}}} - E_{\text{decur}} \right) \quad (12)$$

$$\frac{dIgE}{dt} = k_{\text{in}} \cdot \left(1 - \frac{I_{\text{max}}(1) \cdot C_{\text{ejhl}}}{IC_{50}(1) + C_{\text{ejhl}}} - \frac{I_{\text{max}}(2) \cdot C_{\text{edecur}}}{IC_{50}(2) + C_{\text{edecur}}} \right) - k_{\text{out}} \cdot IgE \quad (13)$$

where C_{ejhl} and C_{edecur} represent the concentrations of JHL45 and decursinol in an effect site; k_{eo1} and k_{eo2} are first-order

processes describing transport of the drug from the central compartment to the effect compartment of JHL45 and decursinol; k_{in} represents the rate of production of IgE; k_{out} defines the first-order rate constant for the loss of IgE; $I_{\text{max}}(1)$ and $I_{\text{max}}(2)$ represent the maximum inhibition effects attributed to JHL45 and decursinol; and $IC_{50}(1)$ and $IC_{50}(2)$ represent the drug concentrations that produce 50% of the maximum inhibition at the effect site.

The disease progression model is quantitative and accounts for the disease status time course and that consists of disease progression and drug action (Krzyszanski & Jusko, 1998). In this model, the change in skin severity score ($S_{(t)}$) depending on lowering of the serum IgE concentration by JHL45 and decursinol was fitted to a linear disease progression model ($N_{(t)}$) with a symptomatic drug effect ($E_{(t)}$) using the following equation:

$$S_{(t)} = S_0 + N_{(t)} + E_{(t)} \quad (14)$$

where $S_{(t)}$ is the skin severity score at time t and S_0 is baseline skin severity score. The linear natural course of disease progression ($N_{(t)}$) can be described as:

$$N_{(t)} = -\alpha \cdot t \quad (15)$$

where α represents the slope of the line representing skin severity score over time in absence of any treatment. The drug effect was modeled using the following E_{max} model:

$$E_{(t)} = \frac{E_{\text{max}} \cdot IgE}{EC_{50} + IgE} \quad (16)$$

where E_{max} is the maximum effect on skin severity score of lowering of the serum IgE concentration by JHL45 and decursinol; EC_{50} represents the serum IgE concentration that produces a 50% maximal effect on skin severity score. The predicted serum IgE concentration at time t , $IgE_{(t)}$, was calculated using Equation (13).

Model fitting was performed by non-linear regression analysis using the maximum likelihood algorithm in ADAPT 5 (D'Argenio et al., 2009). The following information, obtained using ADAPT 5, was used to evaluate the goodness-of-fit and the quality of the parameter estimates: visual representation of the distribution of the residuals, Akaike's information criterion, coefficients of variation for parameter estimates, parameter correlation matrices and sums of squares of residuals. We used a coefficient of variation of <0.5 and a correlation coefficient threshold of 0.9 as the criteria for evaluation of the numerical identifiability of the parameter estimates.

Results

In vitro screening

As shown in Figure 2, JHL45 strongly inhibited IL-6, IL-8 and MCP-1 expression in mite-treated THP-1 and EoL-1 cells without causing cytotoxicity. Indeed, JHL45 suppressed the expression of cytokines at a low concentration (0.1 µg/mL). Dose-dependent inhibition by JHL45 occurred in both THP-1 and EoL-1 cells. Therefore, we evaluated JHL45 as a novel candidate AD drug.

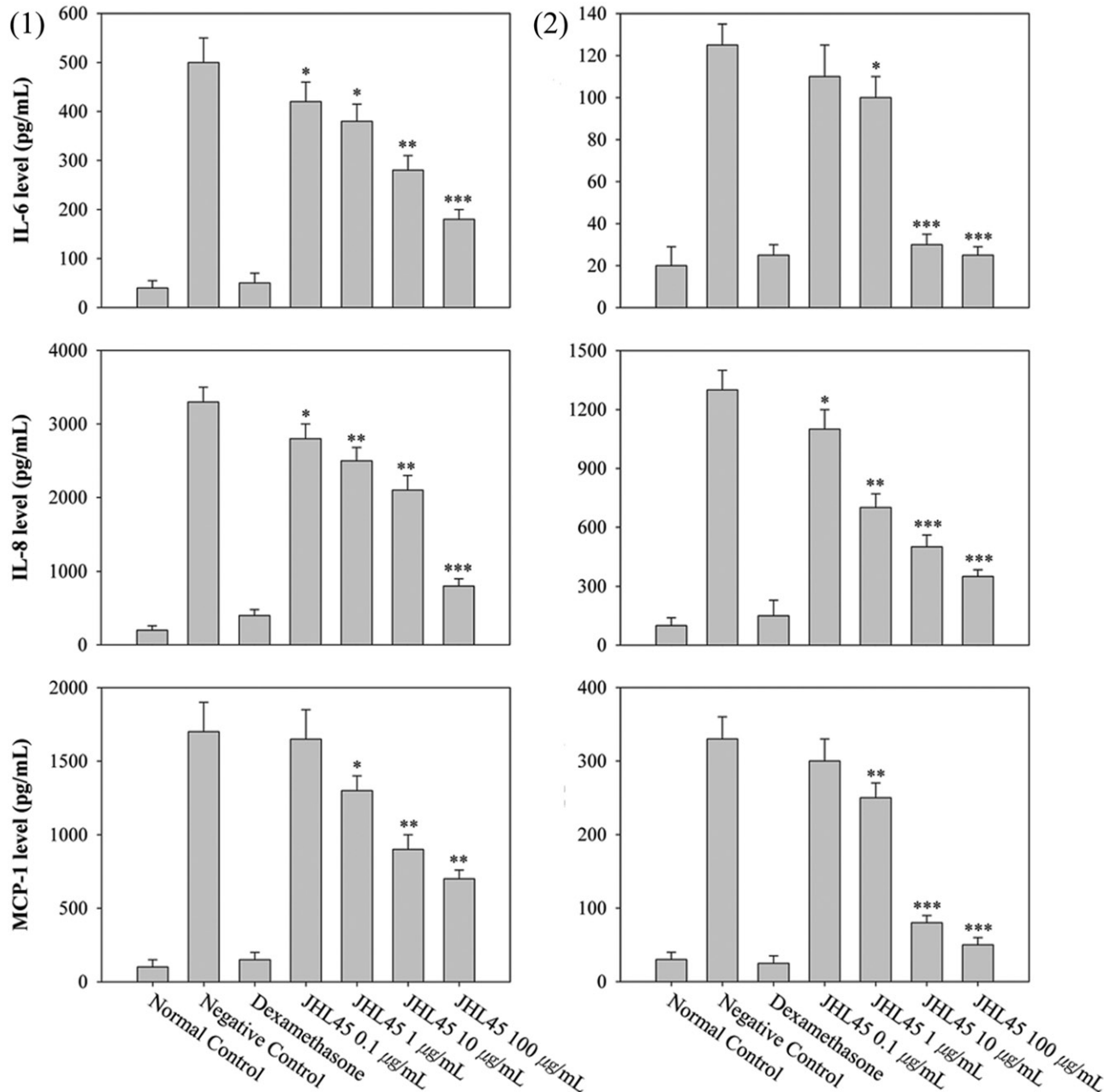


Figure 2. JHL45 inhibits mite extract-induced MCP-1, IL-6 and IL-8 production in THP-1 (1) and EoL-1 (2) cells. Cells treated with mite extract alone were the negative control, and dexamethasone was used as the positive control. Data are presented as means $\pm$ SD of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significant difference between the negative control and the JHL45-treated groups.

Physicochemical properties of JHL45 and decursinol

We investigated the following physicochemical properties of JHL45 and decursinol: kinetic solubility, lipophilicity (log P), ionization (pK_a) and permeability (P_{app}). The results of physicochemical properties of JHL45 and decursinol are given Table 1.

In vitro pharmacokinetic properties of JHL45 and decursinol

Metabolic stability, plasma protein binding and cytochrome P450 inhibition were assessed to characterize the in vitro pharmacokinetic properties of JHL45 and decursinol (Table 2, Figure 3). JHL45 was highly stable in human microsomal

Table 1. Physicochemical properties of JHL45 and decursinol.

Measurement (units)	JHL45	Decursinol
Kinetic solubility ($\mu\text{g/mL}$)	40.43 ± 3.81	123.14 ± 2.89
P_{app} ($\times 10^{-6} \text{ cm/s}$)	0.29 ± 0.21	5.86 ± 0.22
$\log P$	2.64 ± 0.12	2.21 ± 0.79
pK_a	7.08 ± 0.21	11.78 ± 0.24

Data represent the means $\pm$ SD of four experiments.

assays, as $101.04 \pm 5.01\%$ remained after incubation for 30 min at a concentration of $10 \mu\text{M}$. However, it had poor stability ($9.01 \pm 4.76\%$) in rat microsomal assays. Decursinol was highly stable, with $99.98 \pm 10.14\%$ and $90.04 \pm 4.12\%$ remaining after incubation for 30 min in both human and rat

Table 2. *In vitro* microsomal stability and human plasma-protein binding of JHL45 and decursinol.

Measurement (units)	JHL45	Decursinol
Rat liver microsomal stability (%)	9.01 ± 4.76	90.04 ± 4.12
Human liver microsomal stability (%)	101.04 ± 5.01	99.98 ± 10.14
Human plasma protein binding (%)	99.90 ± 0.01	60.70 ± 6.70

Data represent the means ± SD of three experiments.

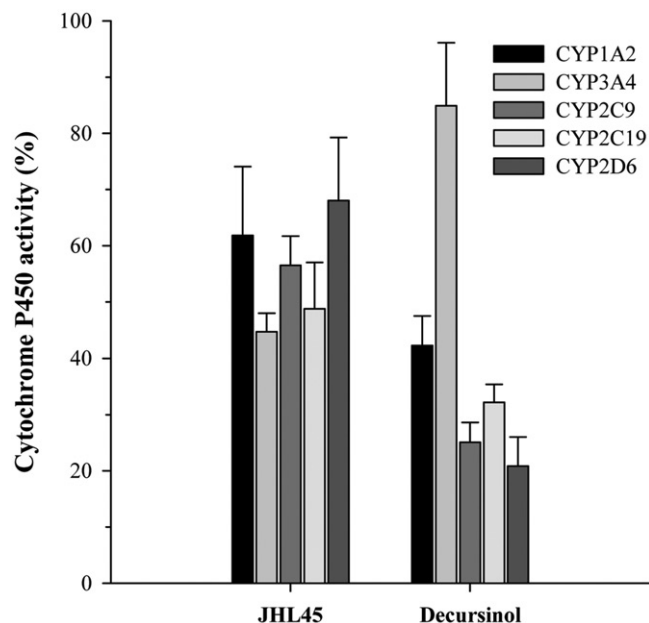


Figure 3. Cytochrome P450 (CYP) activity in the presence of 10 μ M JHL45 and decursinol, as measured using a Vivid® CYP screening kit.

microsomal assays at a concentration of 10 μ M. The protein binding of JHL45 in human plasma was high (99.90 ± 0.01%) after administration at a concentration of 2 μ g/mL; the proportion of decursinol bound to protein (60.70 ± 6.70%) was lower. For JHL45, the remaining activities of 1A2, 3A4, 2C9, 2C19 and 2D6 were 61.83, 44.76, 56.51, 48.79 and 68.03%, respectively. For decursinol, the remaining activities of 1A2, 3A4, 2C9, 2C19 and 2D6 were 42.30, 84.92, 25.09, 32.18 and 20.83%, respectively.

Pharmacokinetics of JHL45 and its metabolite in rats after intravenous and oral administration

The time courses of the mean plasma concentrations of JHL45 and decursinol following intravenous and oral administration of JHL45 (1 and 5 mg/kg) to five SD rats per group are shown in Figure 4. The non-compartmental PK parameters are given in Table 3. After intravenous administration, JHL45 showed rapid elimination, with a half-life of 0.08 to 0.22 h. Also, JHL45 was rapidly metabolized to decursinol, which has a higher exposure (AUC_{8h}) and a longer half-life than JHL45. JHL45 was not detected in plasma following oral administration of JHL45 (1 or 5 mg/kg).

Pharmacokinetic/pharmacodynamic/disease progression model in NC/Nga mice

The mechanism-based PK/PD/DIS model was successfully used to explain the relationship between PK

(plasma concentrations of JHL45 and decursinol), PD (serum IgE concentration) and disease progression (skin severity scores) following repeated oral administration of 1, 3 and 10 mg/kg JHL45 to NC/Nga mice ($n = 10$ per dose group) as follows: an enterohepatic circulation (EHC) model for PK, an indirect response model for PD, and a linear disease progression model ($N_{(t)}$) with a symptomatic drug effect for disease progression. The solid lines in Figure 5 represent the fits of the average observation values, demonstrating that the compartment model and the measured data were consistent. The non-compartmental pharmacokinetic parameters of JHL45 and decursinol on day 1 following single oral administration of JHL45 in NC/Nga mice are given in Table 4. Also, the final estimates of the model-dependent parameters are listed in Table 5.

Discussion

The present study evaluated a novel immune modulator, JHL45, *in vitro* and *in vivo* to describe PK after its IV and PO administration in rats, its effect on IgE concentrations, and the correlation between IgE changes and disease progression in an NC/Nga mice model. Before optimization of PK/PD, the ability of 62 new compounds to inhibit cytokine production by THP-1 human monocytic cells and EoL-1 human eosinophilic cells was investigated, to evaluate their suitability as novel candidate drugs for the treatment of AD. The *in vitro* screening system has been used to identify novel candidate drugs. Notably, suppression of IL-6, IL-8 and MCP-1 in mite-treated THP-1 and EoL-1 cells is a useful *in vitro* screening method for identification of AD drug candidates (Lee et al., 2011). *In vivo* data were used to develop a PK/PD/DIS model as a tool for drug development targeting atopic dermatitis.

First, we investigated the following physicochemical properties of JHL45 and decursinol: kinetic solubility, lipophilicity, ionization and permeability. Solubility is one of the most important physicochemical properties in drug discovery, and particularly the gastrointestinal absorption of orally administered drugs. Generally, the solubility of a compound can be classified as follows: sparingly soluble (<10 μ g/mL), partially soluble (10–100 μ g/mL), and fully soluble (>100 μ g/mL) (Kerns, 2001). According to this classification, JHL45 is a partially soluble compound and decursinol a fully soluble compound. This information is useful for the calculation of maximum absorbable dose (MAD) during development of orally administered drugs (Lipinski, 2000).

The minimum solubility required for oral administration is dependent on the permeability of the lead compound (Di & Kerns, 2003). Compounds with Papp values of $<1 \times 10^{-6}$, $1-10 \times 10^{-5}$, and $>10 \times 10^{-6}$ cm/s are classified as poorly (0–20%), moderately (20–70%) and well (70–100%) absorbed, respectively (Yee, 1997). Therefore, JHL45 is expected to be a poorly absorbed compound and decursinol a moderately absorbed compound. Lipophilicity significantly impacts PK properties and is often expressed as a coefficient of partition ($\log P$) between octanol and aqueous phases. A compound with moderate lipophilicity ($\log P$ 0–3) has a good balance between solubility and permeability and is

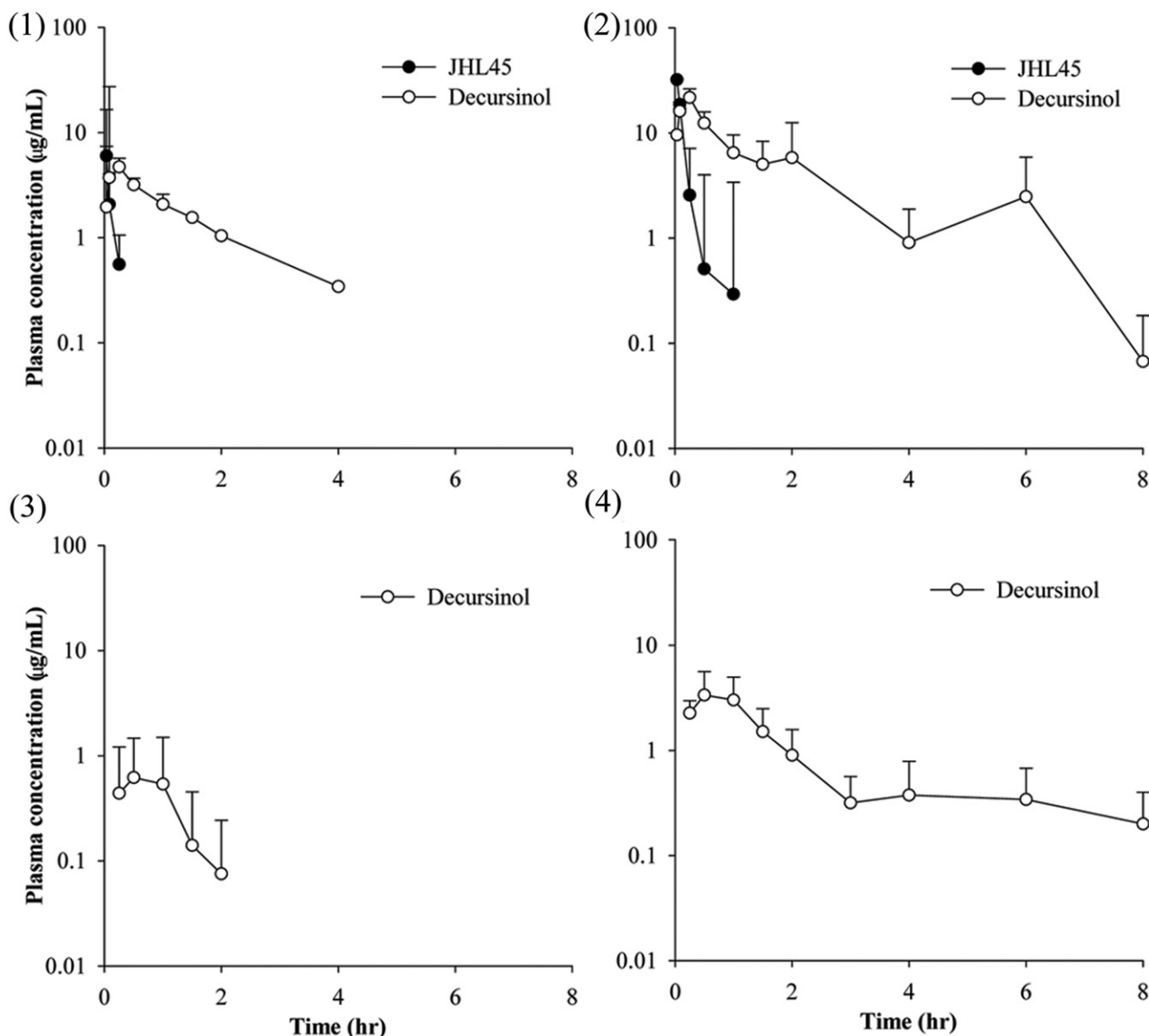


Figure 4. Mean plasma concentration-versus-time profiles of JHL45 (●) and decursinol (○) following intravenous and oral administration of JHL45 (1 and 5 mg/kg) to rats (means $\pm$ SD, $n = 5$). (1) Intravenous administration of JHL 1 mg/kg; (2) intravenous administration of JHL 5 mg/kg; (3) oral administration of JHL 1 mg/kg; (4) oral administration of JHL 5 mg/kg.

optimal for oral absorption (Di & Kerns, 2003). Thus, the results suggest that JHL45 and decursinol are appropriate for oral administration.

pK_a is the ionization constant of a compound, and affects its solubility, permeability, distribution coefficient ($\log D$) and oral absorption by modulating the distribution of neutral and charged species. Basic compounds tend to be more soluble and less permeable at low pH values. Therefore, JHL45 and decursinol may not be ionized in the stomach. Instead, they are mostly ionized in the small intestine, where absorption occurs to a lower degree. pK_a impacts biological activity and metabolism through electrostatic interactions. Information on pK_a facilitates salt-selection processes (Di & Kerns, 2003). A high degree of ionization keeps drugs extracellular and decreases their systemic toxicity. A pK_a value in the range 6–8 is advantageous for membrane penetration. In the present study, JHL45 had a reasonable pK_a , suggesting that it is minimally toxic.

During the early stages of drug development, *in vitro* PK assays play important roles in evaluating drug pharmacokinetics, such as absorption and metabolism. The *in vitro* microsomal stability assay is routinely used for the prediction of intrinsic clearance, a key pharmacokinetic property that influences *in vivo* half-life (Xu et al., 2002). In this study, human and rat liver microsomes were used to assess the extent of the metabolism of JHL45 and decursinol by phase-1 metabolizing enzymes. These data indicate that JHL45 exhibits interspecies differences in terms of microsomal stability. Data from *in vitro* plasma protein-binding experiments that determine the protein-bound drug fraction are frequently used in drug discovery to guide structure design and to prioritize compounds for *in vivo* studies (Smith et al., 2010). Generally, highly plasma protein-bound drugs have a relatively low volume of distribution.

Early information on cytochrome P450 inhibition can be used to develop structure-property relationships and minimize

Table 3. Non-compartmental parameters for the pharmacokinetics of JHL45 and decursinol following intravenous and oral administrations of JHL45 (1 mg/kg and 5 mg/kg) to SD rats.

Dose	Parameter (units)	Intravenous		Oral	
		JHL45	Decursinol (metabolite)	JHL45	Decursinol (metabolite)
1 mg/kg	AUC_{8h} ($\mu\text{g h/mL}$)	0.61 ± 0.19	5.76 ± 0.49	N.A.	1.11 ± 1.47
	AUC_{inf} ($\mu\text{g h/mL}$)	0.69 ± 0.21	7.07 ± 0.86	N.A.	2.85 ± 2.54
	C_{max} ($\mu\text{g/mL}$)	6.03 ± 1.40	4.73 ± 0.24	N.A.	1.03 ± 1.05
	T_{max} (h) ^a	0.03	0.25	N.A.	0.67 ± 0.29
	$t_{1/2}$ (h)	0.07 ± 0.01	1.36 ± 0.50	N.A.	0.34 ± 0.23
	V_{ss} (L/kg)	0.67 ± 0.17	N.A.	N.A.	N.A.
	CL (L/h/kg)	6.11 ± 1.62	N.A.	N.A.	N.A.
5 mg/kg	AUC_{8h} ($\mu\text{g h/mL}$)	3.14 ± 1.41	29.97 ± 17.66	N.A.	5.93 ± 4.01
	AUC_{inf} ($\mu\text{g h/mL}$)	3.62 ± 1.91	30.99 ± 18.21	N.A.	6.88 ± 4.56
	C_{max} ($\mu\text{g/mL}$)	33.25 ± 10.54	21.75 ± 4.59	N.A.	3.50 ± 2.23
	T_{max} (h) ^a	0.03	0.25	N.A.	0.55 ± 0.27
	$t_{1/2}$ (h)	0.45 ± 0.35	1.08 ± 0.68	N.A.	1.23 ± 0.60
	V_{ss} (L/kg)	2.00 ± 1.03	N.A.	N.A.	N.A.
	CL (L/h/kg)	6.47 ± 2.69	N.A.	N.A.	N.A.

Data represent the means $\pm$ SD ($n = 5$); N.A.: not applicable.

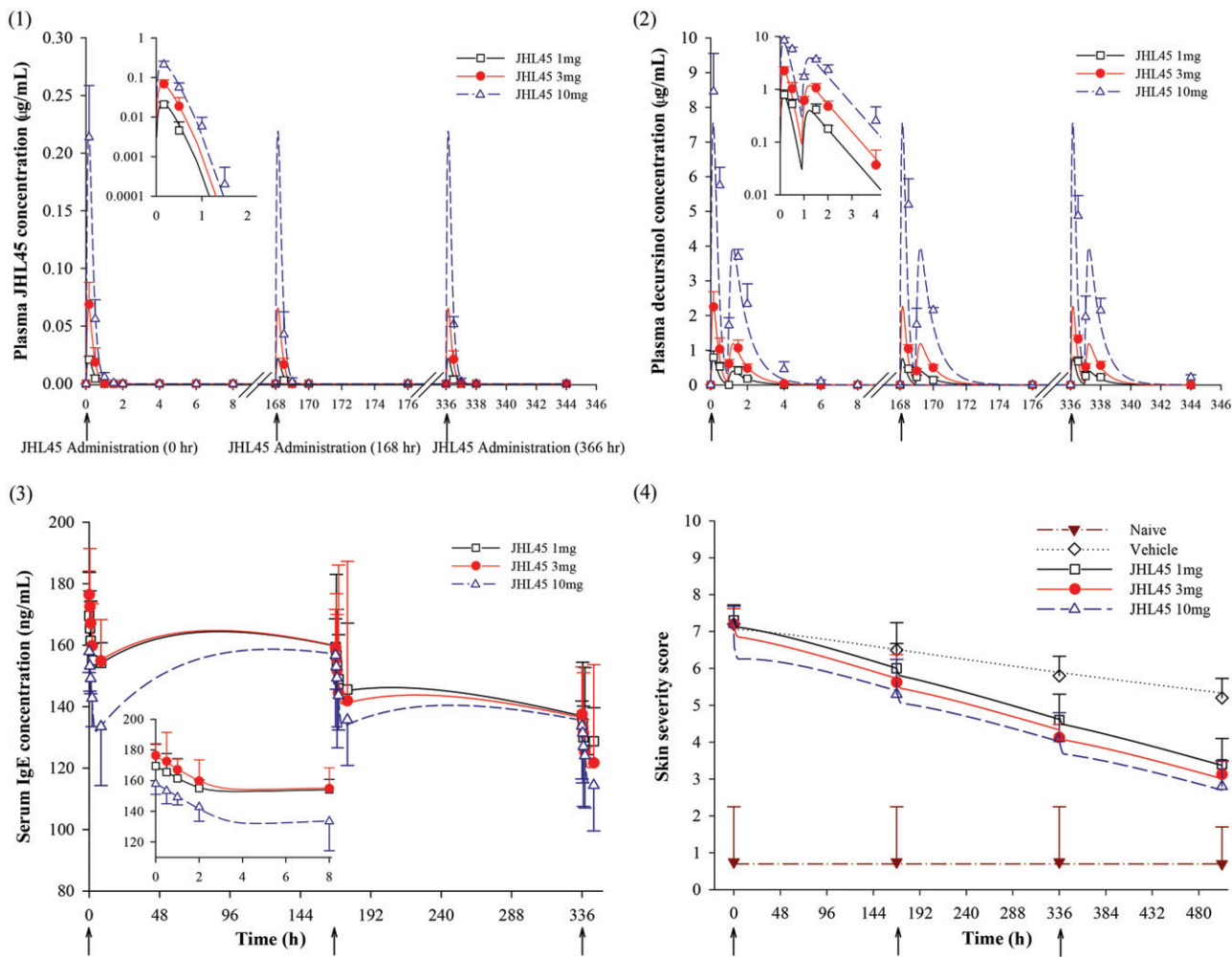


Figure 5. Pharmacokinetics of JHL45 (1) and metabolite (2), pharmacodynamics (3) and disease progression profiles (4) following administration of multiple oral doses of JHL45 (1 mg/kg ($\circ$, black line), 3 mg/kg (Δ , gray line) or 10 mg/kg ($\bullet$, dashed line)) to NC/Nga mice (means $\pm$ SD, $n = 10$). The skin severity scores of normal ($\bullet$, dotted line) and negative control groups ($\bullet$, dash-dotted line) indicate in disease progression profile (4). Each line indicates the final model fits.

Table 4. Non-compartmental parameters for the pharmacokinetics of JHL45 and decursinol on day 1 following oral administration of JHL45 (1, 3 and 10 mg/kg) to NC/Nga mice.

Dose	Parameter (units)	JHL45		Decursinol (metabolite)	
		Mean	SD	Mean	SD
1 mg/kg	AUC_{8h} ($\mu\text{g h/mL}$)	0.63×10^{-2}	0.87×10^{-3}	0.57	0.04
	AUC_{inf} ($\mu\text{g h/mL}$)	0.91×10^{-2}	0.11×10^{-2}	0.61	0.03
	C_{max} ($\mu\text{g/mL}$)	0.02	0.01	0.83	0.02
	T_{max} (h) ^a	0.17	N.A.	0.17	N.A.
	$t_{1/2}$ (h)	0.25	0.03	N.A.	N.A.
3 mg/kg	AUC_{8h} ($\mu\text{g h/mL}$)	0.21×10^{-1}	0.48×10^{-2}	1.70	0.15
	AUC_{inf} ($\mu\text{g h/mL}$)	0.23×10^{-1}	0.46×10^{-2}	1.73	0.14
	C_{max} ($\mu\text{g/mL}$)	0.06	0.01	2.45	0.37
	T_{max} (h) ^a	0.17	N.A.	0.17	N.A.
	$t_{1/2}$ (h)	0.90	0.22	N.A.	N.A.
10 mg/kg	AUC_{8h} ($\mu\text{g h/mL}$)	0.68×10^{-1}	0.63×10^{-2}	5.74	0.17
	AUC_{inf} ($\mu\text{g h/mL}$)	0.74×10^{-1}	0.25×10^{-2}	5.78	0.19
	C_{max} ($\mu\text{g/mL}$)	0.20	0.01	8.51	0.35
	T_{max} (h) ^a	0.17	N.A.	0.17	N.A.
	$t_{1/2}$ (h)	0.16	0.01	N.A.	N.A.

Data represent the means $\pm$ SD ($n = 5$); N.A.: not applicable.

^aMedian; AUC_{8h} , the area under the plasma concentration-versus-time curve from time zero to the last measurable concentration; AUC_{inf} , the area under the plasma concentration-versus-time curve from time zero to infinity; C_{max} , the peak plasma concentration; T_{max} , the time to reach C_{max} ; $t_{1/2}$, the elimination half-life.

the potential toxicity of discovery candidates due to drug–drug interactions. During the early stages of drug discovery, percentage inhibition of clinically relevant CYP450s (e.g. 1A2, 3A4, 2C9, 2C19 and 2D6) is determined at a single concentration (Di & Kerns, 2003). CYP1A2, 3A4, 2C9, 2C19 and 2D6 represent greater than 90% of total hepatic CYP activities, and more than 90% of therapeutics on the market are metabolized by these enzymes (Gao et al., 2002). This result showed that JHL45 has potential clinical relevance for substrates of CYP3A4 and 2C19 (remaining activity <50%). Moreover, decursinol, the major metabolite of JHL45, could have clinically important interactions with CYP2C9 (25.09%), 2C19 (32.18%) and 2D6 (20.83%). Such CYP interaction screening suggests potential drug interactions and facilitates optimization of the lead compound.

The pharmacokinetic profile in rats indicates that JHL45 has poor absorption and is rapidly metabolized to decursinol after oral administration. The physicochemical and *in vitro* PK results suggest possible explanations for the PK of JHL45 and decursinol. First, the poor absorption of JHL45 may be caused due to its low permeability ($0.29 \pm 0.21 \times 10^{-6}$ cm/s). Second, JHL45 may exhibit a first-pass effect due to its poor stability ($9.01 \pm 4.76\%$) in rat microsomal assays.

The mechanistic PK/PD/DIS model was successfully used to explain the relationship between PK (plasma concentrations of JHL45 and decursinol), PD (serum IgE concentration) and disease progression (skin severity scores) following repeated oral administration of 1, 3 and 10 mg/kg JHL45 to NC/Nga mice ($n = 10$ per dose group). Initially, we modeled the PK/PD of JHL45 using different models; however, other models were not sufficient to explain the PK/PD of JHL45 and its metabolite, and provided poor goodness-of-fit. Also, fitting of PK and PD data was performed sequentially as simultaneous fitting did not lead to reliable results.

Table 5. Final parameter estimates from the PK/PD/DIS model in NC/Nga mice.

Section	Parameter (units)	Estimate	CV% ^a
Pharmacokinetics	k_a (h^{-1})	0.76	35.53
	k_{am} (h^{-1})	6.45	29.30
	k_{el} (h^{-1})	1.14	42.98
	k_{elm} (h^{-1})	2.90	24.83
	k_m (h^{-1})	4.93	34.69
	k_{mr} (h^{-1})	16.86	62.10
	V_{jhl} (mL)	338.96	18.73
	V_{decur} (mL)	120.97	38.95
	τ (h)	0.94	12.77
	k_r (h^{-1})	4.15	53.98
Pharmacodynamics	k_{eo1} (h^{-1})	2.05	66.34
	k_{eo2} (h^{-1})	3.22	46.27
	$I_{max}(1)$	3.37	39.17
	$I_{max}(2)$	0.39	61.54
	$IC_{50}(1)$ ($\mu\text{g/mL}$)	0.71×10^{-3}	85.92
	$IC_{50}(2)$ ($\mu\text{g/mL}$)	1.91	67.02
	k_{out} (h^{-1})	0.13×10^{-1}	22.14
	k_{in} (h^{-1})	2.33	22.75
Disease progression	$S_{(0)}$	0.68	20.94
	A (h^{-1})	0.34×10^{-2}	58.51
	E_{max}	7.60	10.26
	EC_{50} (ng/mL)	113.07	15.06

^aCoefficient of variation percentage; k_a , the first-order absorption rate constant for JHL45; k_{am} , the first-order absorption rate constant for decursinol; k_{el} , the first-order elimination rate constant for JHL45; k_{elm} , the first-order elimination rate constant for decursinol; k_m , the formation rate constant of the drug from the JHL45 compartment to the metabolite compartment; k_{mr} , the first-order rate constants for JHL45 and decursinol excreted into bile (the recycling compartment); V_{jhl} , the volume of distribution of JHL45; V_{decur} , the volume of distribution of decursinol; k_{eo1} , first-order process describing transport of the drug from the central compartment to the effect compartment of JHL45; k_{eo2} , first-order process describing transport of the drug from the central compartment to the effect compartment of JHL45; $IC_{50}(1)$, JHL45 concentration that produce 50% of the maximum inhibition at the effect site; $IC_{50}(2)$, decursinol concentration that produce 50% of the maximum inhibition at the effect site; $I_{max}(1)$, the maximum inhibition effect attributed to JHL45; $I_{max}(2)$, the maximum inhibition effect attributed to decursinol; k_{in} , the rate of production of IgE; k_{out} , the first-order rate constant for loss of IgE; k_r , the first-order rate constant for the dispersion of decursinol into the gut compartment at the time of recycling; $N_{(t)}$, the linear natural course of disease progression; $S_{(t)}$, the skin severity score at time t ; S_0 , baseline skin severity score; α , the slope of the line representing skin severity score over time during JHL45 treatment.

The oral bioavailability of JHL45 was higher in NC/Nga mice than in rats. Also, JHL45 was rapidly metabolized to decursinol, which has a double-peak PK in NC/Nga mice. The modeling approaches were developed to explain the double-peak phenomenon in the PK of alprazolam, ranitidine, amprenavir, tesofensine and meloxicam (Lehr et al., 2009; Okusanya et al., 2007; Wang et al., 1999). Notably, drugs undergoing enterohepatic circulation are associated with typical PK characteristics, such as multiple-peak phenomena in the plasma concentration-time profile and prolongation of the apparent elimination half-life ($t_{1/2}$). A quantitative EHC model capable of describing the double peaks in the plasma concentration-time profile of decursinol was successfully developed.

The mechanistic PK–PD models used in this study were based on the indirect response models (Dayneka et al., 1993; Suto et al., 1999). Here, the primary endpoint for PD modeling was a reduction in IgE concentration. The total

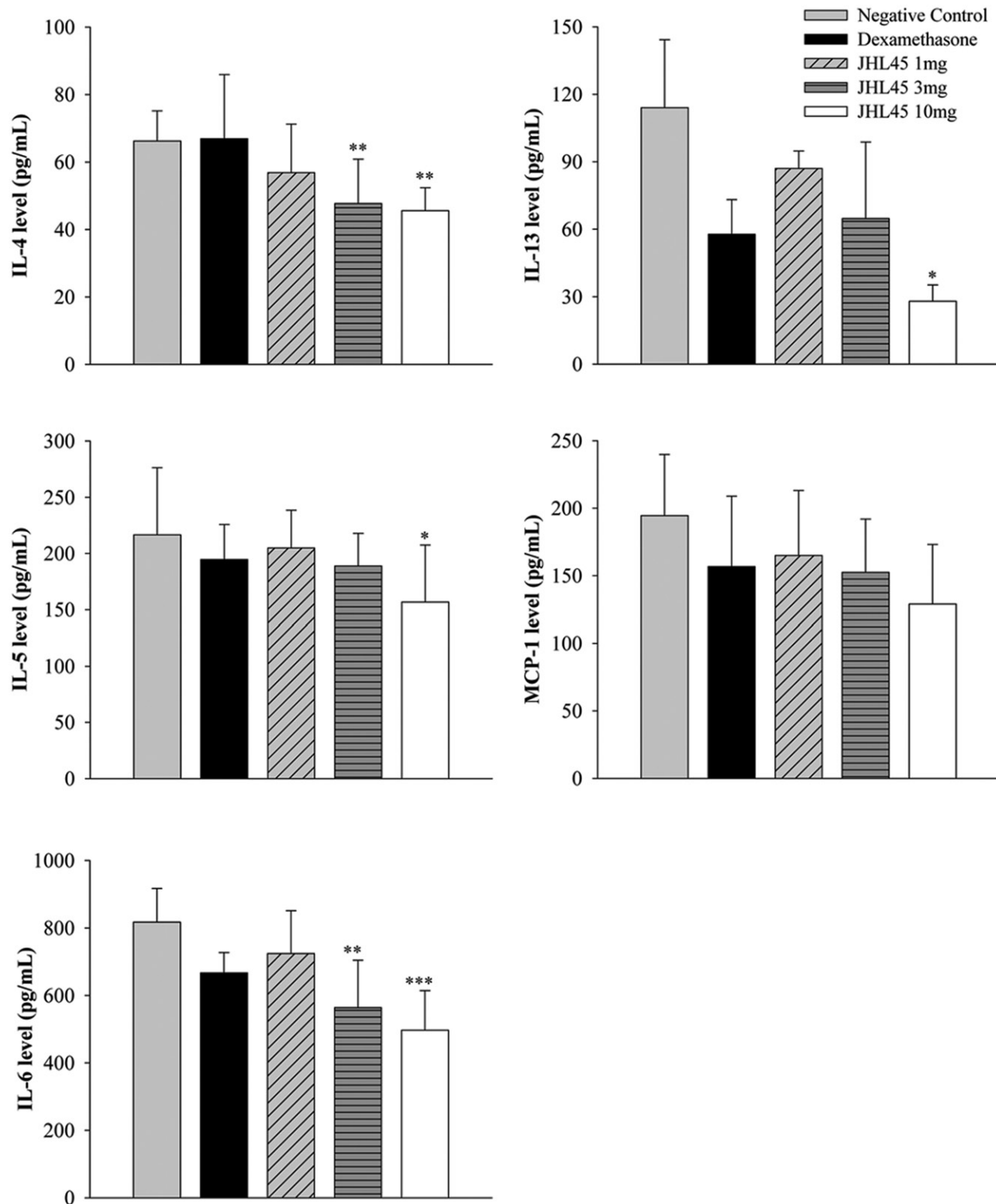


Figure 6. Altered release of cytokines and chemokines from splenocytes from JHL45-treated mice. Data are presented as the means $\pm$ SD of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significant difference between the negative control and the JHL45-treated groups.

serum IgE concentration was decreased by inhibition of the rate of IgE production (k_{in} values), and k_{in} was inhibited by JHL45 and decursinol. Generally, k_{in} is a zero-order rate constant in the indirect response model. In addition, the value of k_{eo} was necessary to explain the delay time to reach plasma-effect compartment equilibrium.

Although the AUC and C_{max} values for JHL45 were lower than those for decursinol, the pharmacological effect of JHL45 ($I_{max}(1) = 3.37$, $IC_{50}(1) = 0.39$ ng/mL) was more potent and efficient than that of decursinol ($I_{max}(2) = 0.71$, $IC_{50}(2) = 1.91$ μ g/mL). The previously described *in vitro* experiments support the PD parameter estimates. The

mechanism-based PK/PD model of JHL45 and decursinol for AD treatment may therefore be a useful approach to the design of new compounds, and provides a scientific basis for extrapolating from *in vitro* pharmacology to *in vivo* studies of the PD of immune modulators for use in AD treatment.

Pharmacological studies in non-clinical and clinical trials focus on testing a null hypothesis because there is an alternative model that can be accepted in its place. Disease progression model-based evaluations provide a basis for developing exposure-response surfaces by making scientifically valid assumptions. Also, disease progression models increase information by turning noise into a signal by providing a basis for the variation. In the present study, the PK/PD model was integrated with a disease progression model to understand the immunopathogenic mechanism of action of JHL45 in AD. We developed a quantitative model that accounts for the disease status time course through skin severity scores for JHL45 and decursinol. The time course of the natural progression of atopic dermatitis (skin severity score) was described using a linear model ($N_{(t)}$) and the drug effect was assumed to be symptomatic for JHL45 and decursinol. Symptomatic effects offset the natural history during treatment, but have no continuing effect after the washout period.

The integration of PK/PD concepts in all stages of preclinical and clinical drug development is a potential approach to information gain and may increase the efficiency of the decision-making process during drug development. The PK/PD/DIS model is a more advanced mechanistic PK/PD model. This modeling approach could be used to characterize the PK and PD of JHL45 and its metabolites, and possibly to determine the mechanisms of action drugs. Implementation of optimal evaluation in animal models of AD and PK/PD/DIS modeling is a novel approach that holds promise for improving and accelerating the understanding of the action of immunomodulatory compounds *in vivo*. To determine the effects of JHL45 and decursinol in DNCB-treated Nc/Nga mice, splenocytes were isolated from the spleens of mice and then treated. The release of IL-4, IL-5, IL-6 and IL-13 was down-regulated in splenocytes from JHL45-treated mice compared with the control group. In addition, the secretion of MCP-1, a potent monocyte chemo-attractant, was found to be down-regulated in the JHL45 compared to the control group (Figure 6). These results indicate that JHL45 decreases IgE production by regulating Th1 and Th2 cytokine production.

Conclusion

Our findings indicate that JHL45 has good physicochemical properties and powerful pharmacological effects when administered orally for treatment of AD in rodents. The PK/PD/DIS model described well the rapid metabolism of JHL45, the double-peak phenomenon in the PK of decursinol, and the inhibition of IgE generation by compounds in NC/Nga mice. Also, AD disease progression was successfully characterized using a quantitative model of the complex interactions between serum IgE concentration and skin symptoms in AD. These findings have important implications for AD drug development.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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