Cutting Edge: Selective Oral ROCK2 Inhibitor Reduces Clinical Scores in Patients with Psoriasis Vulgaris and Normalizes Skin Pathology via Concurrent Regulation of IL-17 and IL-10


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Targeted inhibition of Rho-associated kinase (ROCK)2 downregulates the proinflammatory T cell response while increasing the regulatory arm of the immune response in animal models of autoimmunity and Th17-skewing human cell culture in vitro. In this study, we report that oral administration of a selective ROCK2 inhibitor, KD025, reduces psoriasis area and severity index scores by 50% from baseline in 46% of patients with psoriasis vulgaris, and it decreases epidermal thickness as well as T cell infiltration in the skin. We observed significant reductions of IL-17 and IL-23, but not IL-6 and TNF-α, whereas IL-10 levels were increased in peripheral blood of clinical responders after 12 wk of treatment with KD025. Collectively, these data demonstrate that an orally available selective ROCK2 inhibitor downregulates the Th17-driven autoimmune response and improved clinical symptoms in psoriatic patients via a defined molecular mechanism that involves concurrent modulation of cytokines without deleterious impact on the rest of the immune system. The Journal of Immunology, 2017, 198: 000–000.

Psoriasis is one of the most common immune-mediated skin disorders characterized by a dysregulated IL-23/Th17-dependent immune response (1–3). Psoriatic skin lesions contain increased infiltrates of activated innate immune cells producing IL-23, which induces Th17 subset of T cells secreting IL-17A and IL-17F (4, 5). These proinflammatory cytokines can activate keratinocytes and endothelial cells in the skin by binding to the IL-17 receptor, leading to development of skin inflammation and disease progression (6, 7). Therefore, a number of biologic agents targeting the IL-23/Th17 pathway have demonstrated robust efficacy in the clinic and been approved recently for the treatment of moderate to severe plaque psoriasis (8–10). However, the use of aforementioned biologics for psoriasis and other chronic autoimmune disorders can be limited by long-term safety issues, such as an increase rate of infections due to the central role of IL-17 in protection against Candida (8–10). Additionally, biologic therapies are costly compared with traditional systemic therapy and are administered via injection, which may limit patient acceptability (11, 12). Thus, the development of new oral therapies remains important and can offer an alternative risk-benefit profile via modulation of specific disease-associated cellular pathways.

Rho-associated kinase (ROCK)2 was shown recently to be implicated in the regulation of autoimmunity in mice and humans (13–15). Previous findings demonstrated that oral administration of a selective ROCK2 inhibitor (KD025) in healthy subjects decreases IL-17 and IL-21 secretion induced by ex vivo stimulation (15). Moreover, targeted ROCK2 inhibition shifted the balance between proinflammatory and

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The online version of this article contains supplemental material. Abbreviations used in this article: K16, keratin 16; PASI, psoriasis area and severity index; PASI 50, 50% decrease in PASI; PASI 75, 75% decrease in PASI; ROCK, Rho-associated kinase; ROR, retinoic acid–related orphan receptor.

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immunosuppressive T cell subsets via concurrent regulation of STAT3/STAT5 phosphorylation (16–18). Thus, we have hypothesized that targeting of ROCK2 may restore disrupted immune homeostasis and could have a role in the treatment of Th17-mediated inflammatory disorders such as psoriasis.

Materials and Methods

Study design

KD025 (formerly Sfx-2119) is ATP competitive and 100-fold more selective for the ROCK2 isoform compared with ROCK1, with no significant activity against 300 intracellular kinases and surface receptors (15, 19). A phase 2, open-label, dose-finding study was initiated to evaluate the safety, tolerability, and activity of KD025 in subjects with psoriasis vulgaris (NCT02317627 at ClinicalTrials.gov). This study enrolled subjects, between the ages of 18 and 65 y, diagnosed with moderately severe plaque psoriasis who had been stable for at least 4 wk prior to study entry and had failed at least one systemic or phototherapy who had at least 10% of body surface area affected by plaque psoriasis and a psoriasis area and severity index (PASI) of ≥12 within the 24 h period prior to the first dose of study drug; and who were not on any concomitant systemic and topical therapy during the course of the study. Patients treated with immunosuppressive agents and biologic therapies within 4 wk and 56 wk, respectively, prior to study entry were excluded. In the study, which was conducted at eight sites in the United States, 38 patients were randomized and orally received KD025 at three daily dose regimens for 12 wk: 13 subjects received 200 mg twice a day, 13 subjects received 400 mg once a day, and 12 subjects received 400 mg twice a day. Safety and efficacy parameters were analyzed throughout the entire study. Safety evaluations included adverse event assessment, vital sign measurements, electrocardiographic assessments, clinical laboratory tests, and physical examinations. PASI scores were measured on a monthly basis: predose, end of 4 wk, end of 8 wk, end of 12 wk, and 30 d after the last dose of KD025 (30-d follow-up visit). All patients provided written informed consent, and Institutional Review Boards/Ethics Committees approved the protocol.

Skin biopsy specimens and histological analysis

Skin punch biopsies from clear unaffected skin and a selected lesion were collected from some patients before the treatment with KD025 and from the selected lesion on the last day of 12 wk (at the end of the treatment). Biopsied tissues were evaluated by using standard ABC protocol for immunohistochemistry staining with H&E, and Abs against keratin 16 (K16; MCA1868, 1:200; AbD Serotec), CD3 (349201, 1:100; BD Biosciences), pSTAT3 (4113, 1:25; Cell Signaling Technology), pSTAT5 (4322, 1:25; Cell Signaling Technology), retinoic acid-receptor orphan receptor (ROR)γt (14-6988, 1:25; Affymetrix/eBioscience), IRF4 (sc-48338, 1:10; Santa Cruz Biotechnol- ogy), ROCK1 (HPA007567, 1:100; Sigma Life Science), and ROCK2 (HPA007459, 1:100; Sigma Life Science). The number of positive cells per square millimeter of epidermis/dermis was counted manually by using National Institutes of Health Image 6.1 software (CD3, pSTAT3, pSTAT5, and IRF4 staining). RORγt, ROCK1, and ROCK2 expression was defined by quantitative analysis that was converted into numerical quantification units: 0, negative; 0.5, negative/positive; 1, positive; and 2, positive/positive. Additional skin biopsies were processed, and 10-μm thick paraffin wax sections were cut and stained with hematoxylin and eosin (H&E) and Abs against 300 intracellular kinases and surface receptors (15, 21). Immunofluorescence staining was performed on OCT-frozen skin tissue samples (stored at −80°C) by dissolving and washing in PBS, then lyzed in RIPA buffer (25 mM Tris-HCl [pH 7.6], 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS) in the presence of protease inhibitor mixture (Roche) by two cycles of freezing and thawing. Sample buffer was then added, and, after boiling, the samples, containing equal amounts of proteins, were separated on an SDS-PAGE gel and transferred to nitrocellulose membrane.

Peripheral blood sample collection and cytokine analysis

Pharmacokinetic analysis was performed at selected study sites, and peripheral blood samples were collected from a subgroup of subjects from each cohort at the following time points: predose (time 0) and 1, 2, 4, 6, 8, 12, 16, 18, and 24 h after dosing on day 1 of month 1. For cytokine evaluation, peripheral blood samples were collected from all patients at baseline (predose), after 4, 8, 24 h after dosing on day 1 of month 1. 12 wk of treatment with KD025, and at the follow-up visit, that is, 30 d after the last dosing with the drug. IL-17A, TNF-α, and IL-6 were measured by three-plex B, whereas IL-10 and IL-23 were detected by single-plex assays kits by using the Quanterix Simoa ELISA-based platform for biomolecule detection (20). The frozen plasma samples were shipped and tested in duplicates at PBL. Article: Asay Service (Piscatway, NJ).

PBMCs were isolated by spinning samples collected into BD Vacutainer cell preparation tubes (362753; BD Biosciences) and frozen at −80°C in a solution of 10% DMSO and 90% FBS using a Nalgene Cryo 1°C freezing container (Nalgene 1000-0001). Cells were then thawed and washed with 37°C complete RPMI 1640 and rested overnight in an incubator at 37°C. Cells were counted, and for cytokine staining cells were stimulated with PMA/ionomycin and GolgiStop for 4 h, washed and stained with a viability dye (65-0865; eBioscience), washed again with flow cytometry staining buffer (00-4222; eBioscience), fixed, and permeabilized (00-5521; eBioscience), and then stained for CD4 (45-0049; eBioscience), IL-17A (11-7179; eBioscience), and IL-21 (50-7219; eBioscience). For the transcription factor staining, cells were left unstained and stained with a viability dye and CD4, washed, fixed, and permeabilized, and then stained for FOXP3 (25-4777; eBioscience). Samples were analyzed on a Millipore Guava 8HT six-color flow cytometer using appropriate single color controls and compensation using OneComp eBeads (01-1111; eBioscience). Flow cytometry files were analyzed using FlowJo version 10.6.7. Charts from data were generated using GraphPad Prism version 7.01.

Results and Discussion

We conducted a phase 2, open-label, dose-finding study to evaluate the safety, tolerability, and activity of KD025 in subjects with psoriasis vulgaris who have failed at least one line of systemic or phototherapy (NCT02317627 at ClinicalTrials.gov). KD025 was orally administered at three dosage regimens: 200 mg twice a day, 400 mg once a day, and 400 mg twice a day for 12 wk. Of a total of 38 patients dosed in the study (Supplemental Fig. 1A), 26 patients completed the study, including 7 in the 200 mg twice a day cohort, 12 in the 400 mg once a day cohort, and 7 in the 400 mg twice a day cohort (Fig. 1A). Twelve patients were discontinued: one was due to lost follow up, four subjects withdrew voluntarily, and seven subjects (three in the 200 mg twice a day cohort and four in the 400 mg twice a day cohort) were due to grade 2–3 elevations in liver transaminases (alanine aminotransferase and aspartate aminotransferase) with no increase in bilirubin levels. All transaminase elevations were reversible and returned to normal when drug treatment was stopped. In general, KD025 was orally available and well tolerated, and no serious adverse events related to treatment were reported in any of the three cohorts (Supplemental Fig. 1B, 1C).

To evaluate the therapeutic potential of a selective ROCK2 inhibitor, PASI scores were measured on a monthly basis during the study. At baseline, the mean PASI scores were comparable between all three cohorts (Supplemental Fig. 1D). A total of 22 (85%) patients who completed the trial demonstrated a clinical benefit, which is defined as any decrease from the baseline PASI score, and 46% of patients achieved at least a 50% decrease in PASI score (PASI 50). The PASI scores decreased gradually over time: 14, 29, and 71% of patients in the 400 mg once a day cohort achieved PASI 50 after 4, 8, and 12 wk of KD025 treatment, respectively (Fig. 1B). At week 12, 42% of patients in the 400 mg once a day cohort and 29% of patients in the 400 mg twice a day cohort achieved a PASI 50 response (Fig. 1B), suggesting a potential clinical benefit of using a lower dosage regimen (200 mg twice a day) of the selective ROCK2 inhibitor KD025, which is 75–100-fold more selective for ROCK2 than for ROCK1 (15, 21). Interestingly, a recent study demonstrated that inhibition of both ROCK1 and ROCK2
isoforms with pan-ROCK inhibitors potently induces proliferation of primary human keratinocytes in vitro, and might have a negative impact in proinflammatory settings such as psoriasis compared with selective ROCK2 inhibition (22). A 75% decrease in PASI (PASI 75) response was achieved in four patients: one in the 200 mg twice a day, two in the 400 mg once a day, and 400 mg twice a day for 12 wk, and clinical response was defined by changes in PASI scores. Percentages of patients achieving a PASI 50 response in each cohort and representative images are shown (B). Histologic evaluation of skin thickness (H&E), keratinocyte proliferation (K16), and T cell infiltrate (CD3) of nonlesional skin (NS) and lesional (LS) skin in 13 patients before (month 1 day 1 [M1D1]) and after (month 3 day 29 [M3D29]) the treatment with KD025 (C). Representative images (original magnification ×10) as well as average data ± SEM are shown. The p values were calculated by a paired t test.

We performed histological analysis of skin biopsy specimens collected and available from 13 patients (from all three cohorts) before initiation (month 1 day 1) and after 12 wk of treatment with KD025 (month 3 day 29). Before treatment, epidermal hyperplasia demonstrated by H&E and K16 staining as well as T cell counts (CD3 staining) were increased in lesional skin (month 1 day 1) compared with values in nonlesional skin (month 1 day 1). KD025 significantly decreased overall epidermal thickness, K16 expression, and the T cell infiltrate in both epidermis and dermis compartments after 12 wk of treatment (Fig. 1C).

It was recently reported that IL-17 levels are increased in the lesional skin and peripheral blood of patients with psoriasis and
correlate with disease severity. Additionally, several groups demonstrated that ROCK2 specifically contributes to regulation of IL-17 secretion in mice and humans (13, 15). To explore whether plasma IL-17 levels could be used to monitor response to the KD025 treatment, peripheral blood samples were collected on a monthly basis and cytokine levels were measured by using a Quanterix Simoa platform for biomolecule detection. We found that KD025 significantly reduced

**FIGURE 2.** Treatment with KD025 downregulates IL-17/IL-23 while increasing IL-10 levels in psoriatic patients. Peripheral blood samples were collected from all patients on a monthly basis. IL-17, IL-23, and IL-10 levels were determined by using the Simoa immunoassay. (A and B) Average data ± SEM from 22 patients who completed the treatment and responded clinically are shown. (C and D) Percentage change was calculated as \((\text{value during treatment} / \text{predose value}) \times 100\%\). (E) Intracellular staining for FOXP3 was performed in PBMCs from 12 patients who completed the study and achieved a PASI 50 clinical response. The \(p\) values were calculated by a paired \(t\) test.

**FIGURE 3.** Targeted ROCK2 inhibition downregulates the expression of Th17-associated molecules, such as ROCK2, pSTAT3, and RORSyt, in lesional skin of psoriasis patients. Representative images (original magnification \(\times 10\)) (A) and average data ± SEM (B) of pSTAT3, RORSyt, ROCK1, and ROCK2 staining from nonlesional and lesional skin in 13 patients before and after the 12 wk treatment with KD025 are shown. The \(p\) values were calculated by a paired \(t\) test.
both IL-17 and IL-23 levels in a time-dependent manner in patients who completed the study and responded clinically to the treatment (Fig. 2A). Whereas there was no change in TNF-α and IL-6 levels (Fig. 2B), KD025 significantly increased levels of immunosuppressive cytokine IL-10 in psoriatic patients after 12 wk of the treatment (Fig. 2A). The upregulation of IL-10 secretion as well as increase in the percentage of FOXP3+ cells was also demonstrated in purified human CD4+ T cells treated with KD025 during Th17-skewing conditions in vitro (15). The levels of IL-17 and IL-10 returned to the baseline (pseudose values), but IL-23 levels remained diminished even 30 d after discontinuation of the treatment with KD025 (30-d follow-up). There was a correlation between changes in PASI and IL-17 peripheral blood levels (Fig. 2C), confirming the critical role of IL-17 in psoriasis pathogenesis. Although we did not observe a correlation between changes in IL-23/IL-10 and the clinical response across different patients, some clinical responders demonstrated concurrent downregulation of PASI scores, IL-17, and IL-23, and an increase of IL-10 over time (Fig. 2D, Supplemental Fig. 2A, 2B). Additionally, the intracellular analysis performed in PBMCs isolated from 12 patients who completed the study and achieved PASI 50 clinical response revealed that after 12 wk of treatment, KD025 significantly increased the percentage of FOXP3+ (Fig. 2E) and concurrently reduced the percentage of IL-17 CD4+ T cells (Supplemental Fig. 2C), further demonstrating the immunomodulatory potential of selective ROCK2 inhibition in psoriasis patients.

ROCK2 is induced during Th17-skewing conditions and regulates IL-17 secretion via a STAT3/IRF4/RORyt-dependent mechanism in mice and humans (13, 15–17). Histological assessment revealed that baseline pSTAT3, IRF4, RORyt, and ROCK2 staining were greater, whereas ROCK1 expression was diminished in lesional skin in comparison with nonlesional skin (Fig. 3, Supplemental Fig. 2D). Selective ROCK2 inhibition decreased pSTAT3+ cells in epidermis, but not in dermis, and it reduced the intensity of RORyt and ROCK2 staining after 12 wk of treatment with a minimal effect on the expression of ROCK1 (Fig. 3, Supplemental Fig. 2D). Additionally, numbers of IRF4+ and pSTAT5+ cells were modestly decreased in the dermal, but not epidermal, lesion skin (Supplemental Fig. 2E). Collectively, these data demonstrate that KD025-mediated improvement of clinical scores and skin pathology was associated with downregulation of key molecules implicated in the regulation of the Th17 pathway, such as ROCK2, pSTAT3, RORyt, and IRF4, further defining the molecular mechanism of action of a selective ROCK2 inhibitor in psoriatic patients.

In conclusion, these results demonstrate, to our knowledge for the first time, that oral administration of the selective ROCK2 inhibitor improves clinical symptoms in patients with psoriasis vulgaris and attenuates Th17-driven autoimmune response via concurrent modulation of pro- (IL-17/IL-23) and anti-inflammatory (IL-10) cytokines. In agreement with previous findings in animal models and Th17-skewing in vitro culture of human T cells (15–17), this study further confirmed the potential of selective ROCK2 inhibition to downregulate autoimmune-driven pathology via shifting the balance between proinflammatory and anti-inflammatory immune cell responses. Although the clinical improvement achieved in the same time span by treatment with KD025 was not as robust as the response seen with other oral treatments, such as methotrexate, JAK inhibitors, and apremilast, or newer biologics neutralizing cytokines in the Th17 pathway, future randomized trials of KD025 carried out for a longer time period (to account for a different molecular mechanism of action) in a larger population of psoriatic patients are required to further validate the therapeutic potential and risk-benefit profile of targeted ROCK2 inhibition in psoriasis.

Disclosures
The authors have no financial conflicts of interest.

References

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