

Evaluation of the effect of the specific CCR1 antagonist CP-481715 on the clinical and cellular responses observed following epicutaneous nickel challenge in human subjects

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Background: The CC-chemokine receptor-1 (CCR1) is thought to be involved in recruitment of inflammatory cells in allergic contact dermatitis (ACD). CP-481715 is a specific antagonist of CCR1.

Objectives: To determine the inhibitory effects of CP-418 715 in ACD by evaluating the clinical signs and cellular infiltration in skin biopsies following epicutaneous nickel challenge in allergic subjects.

Subjects/Methods: In this phase 1/2 study, 40 subjects were randomized to 5 days of treatment in four parallel groups (placebo three times daily (TID), placebo once daily (QD), 1000 mg CP-418 715 TID, and 3000 mg CP-418 715 QD). Twenty-four hours after the first drug administration, nickel sulfate patches were applied on subjects' backs and removed 48 hours later.

Results: Pretreatment with 1000 mg CP-481715 TID resulted in significant reductions in visual scores of the nickel reactions ($P = 0.01$). Instrumentally measured erythema tended to decrease in the CP-481715 mg TID group ($P = 0.06$). No differences were noted between the 3000 mg CP-481715 mg QD group and pooled placebo. No significant differences were found for immunohistological cell counts. CP-418 715 was generally safe and well tolerated.

Conclusions: Blocking of CCR1 only partly inhibited clinical manifestations of ACD. Several chemokine receptors are likely relevant for the cellular influx observed in ACD lesions.

Key words: allergic contact dermatitis; CC-chemokine receptor-1 antagonist; chemokines.
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Allergic contact dermatitis (ACD) is an inflammatory skin condition that develops as a consequence of exposure to a hapten exceeding an individual threshold concentration in a contact-sensitized individual. Cross-sectional studies in Denmark from 1990 and 1998 show that ACD is a common disease with an increasing incidence (1). One of the more reproducible contact allergens is nickel, as epicutaneous challenge by nickel sulfate has led to qualitatively similar clinical responses in 90–95% of tests (2). The model of cutaneous nickel allergy has been used to test different therapeutic agents,

including corticosteroids (3) and pimecrolimus (4). The underlying mechanism of ACD is a delayed type hypersensitivity reaction in which the activation of allergen-specific CD4⁺ and CD8⁺ lymphocytes stimulates recruitment of other leucocytes that induce an inflammatory response in the skin. Chemokines are a large family of small (8–10 kDa) chemotactic cytokines that play an important role in the selective recruitment and in the activation of leucocytes to inflammatory lesions. The pro-inflammatory role of chemokines in allergic, inflammatory, and autoimmune

diseases, such as allergic asthma, rheumatoid arthritis, and multiple sclerosis has been documented in several preclinical and clinical studies (5, 6). Several chemokines have also been shown to be involved in the recruitment of inflammatory cells in ACD (7).

Chemokines are divided into four subgroups (CXC, CC, C, and CX3C) based on the position of two conserved cysteine residues on the N-terminus. The specific actions of chemokines are mediated by their specific receptors that belong to the G-protein-coupled receptor family and are expressed on a wide variety of immune cells. One chemokine receptor thought to play a crucial role in several diseases is the CC-chemokine receptor-1 (CCR1). CCR1 is expressed on monocytes, T-cells, dendritic cells, and neutrophils, and interacts with at least eight different CC chemokines: CCL3 (macrophage inflammatory protein-1 α), CCL5 (regulated on activation normal T-cell expressed and secreted, RANTES), CCL7 (monocyte chemotactic protein (MCP)-3), CCL14 (haemofiltrate C-C chemokine-1), CCL8 (MCP-2), CCL15 (leukotactin-1), CCL23 (myeloid progenitor inhibitory factor-1), and CCL16 (haemofiltrate C-C chemokine-4).

The use of specific antagonists to chemokine receptors is a reasonable approach to limit leucocyte infiltration and activation. CP-481715 (quinoxaline-2-carboxylic acid [4(R)-carbamoyl-1(S)-(3-fluorobenzyl)-2(S), 7-dihydroxy-7-methyloctyl]amide) is a specific, small-molecular-weight antagonist of CCR1. CP-481715 behaves as a competitive and reversible antagonist and is selective for CCR1. Evidence for its potential use in human disease was suggested by its ability to inhibit 90% of the monocyte chemotactic activity present in 11/15 rheumatoid arthritis synovial fluid samples (8). CP-418 715 was also shown to inhibit *ex vivo* stimulation of whole blood by CCL3 in healthy volunteers after oral administration and was well tolerated at doses up to 3000 mg (9). Furthermore, in clinical trials conducted in rheumatoid arthritis patients, CP-481715 was able to significantly decrease the overall cellularity and the number of macrophages and CCR1⁺ cells in the synovium compared with samples from placebo-treated patients (10). However, no clinical improvements were detected in rheumatoid arthritis patients (11). CP-481715 has selectivity for the human CCR1 receptor, preventing its evaluation in animal models of human disease. Therefore, transgenic mice expressing the human CCR1 receptor were used to assess the delayed type hypersensitivity reaction in sheep red blood cell-sensitized mice. When administered as a single injection at the time of rechallenge, CP-481715 significantly

inhibited footpad swelling and decreased the amount of IFN- γ and IL-2 produced by isolated spleen cells from sensitized animals (12).

The purpose of the present study was to determine the effect of CCR1 antagonists on the clinical and histological features of ACD. Specifically, the primary study objectives were to evaluate the pharmacodynamic effects of CP-481715 on (i) clinical signs as measured by clinical scoring and instrumental measurement of erythema and (ii) the cellular infiltration in skin biopsies following epicutaneous contact allergen challenge with nickel sulfate in nickel allergic subjects. In addition, investigation of the safety, tolerability, and pharmacokinetics (PK) of CP-481715 was planned.

Subjects and Methods

Design

The study was designed as a phase 1/2, placebo-controlled, randomized, double-blind, parallel group, multiple-dose study to evaluate the effects of CP-481715 on clinical responses and cellular infiltration following nickel challenge in allergic subjects. One study centre in Denmark and four centres in the USA participated in the study.

Ethical considerations

The study was approved by the local Regulatory Authorities and Independent Ethics Committees. Informed consent was obtained from all participating subjects prior to study entry. The study was conducted in accordance with the relevant articles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines.

Study population

Normal, healthy subjects, male and female, of skin phototype I–III (or up to phototype V for US sites), and aged 18–55 years inclusive, were recruited for the study. Subjects had a history of nickel allergy, confirmed by a visual positive reaction (graded at least ++) to nickel sulfate TRUE TEST[®] (Allerderm, Phoenix, AZ, USA) at two skin sites performed during the screening visit. For US sites, women of child-bearing potential had to use 2 reliable methods of contraception from the screening visit until completion of the follow-up visit. For the European site, female subjects were women of no child-bearing potential or women who had undergone documented tubal ligation. Subjects with a history of recent infections/traumas/ultraviolet-irradiation

or use of topical or systemic treatments were excluded.

Screening

Subjects with a clinical history of cutaneous nickel allergy were screened using a standard nickel sulfate patch test (TRUE TEST). An instrumental measure of erythema using a daily calibrated reflectance chromameter (Minolta Chromameter CR-300/CR-310, Solna, Sweden) was performed at one nickel sulfate test site (L1p) and one non-patched, negative control site (L1np) on the *left* upper back (paraspinal region) of each subject just prior to patch application. Three readings were taken over the centre of each of the designated sites at each time-point. The machine-calculated average of the three readings was recorded and used for analysis of the data. Two nickel sulfate patches were applied on the skin of the designated region (L1p and L2p) for approximately 48 hours. Approximately 1 hour following the patch removal, both skin sites were graded visually according to the criteria of the International Contact Dermatitis Research Group (13). Subjects were required to have a clinical score of ++ at both L1p and L2p in order to be eligible for the study. Eligible subjects continued to have evaluations by clinical visual scores (L1p, L2p) and instrumental measurements of erythema (L1p, L1np) at 48 + 1, 72 and 144 hours after patch application. A 4-mm biopsy was taken from L2p at 48 + 1 hours.

Study treatment

At least 21 days after completion of the screening visit, eligible subjects were assigned according to a computer-generated randomization program into four parallel treatment groups: group 1: placebo three times daily (TID); group 2: placebo once daily (QD); group 3: 1000 mg CP-481715 TID; and group 4: 3000 mg CP-481715 QD. Subjects were treated for a total of five consecutive days. The study medication was supplied as an oral powder for constitution in bottles, which was constituted with a vehicle solution prior to administration. The test drug has a bitter taste by nature. In order to maintain the blinding, the bitterness was reproduced by adding denatonium benzoate to the placebo. The appropriate dose of CP-481715 or placebo was administered with 240 ml of water. A standard FDA high-fat breakfast was provided within 30 minutes prior to the morning dose from D1 to D5 as a previous study had shown a significantly increased systemic exposure of CP-481715 when

the dose was administered after a high-fat meal (9). Subjects were instructed to have a high-fat lunch.

Study period

D1. A 4-mm skin biopsy was taken from a non-patched control site (R4np) on the right lower back prior to the first dosing. Study medication or placebo was administered on a QD or TID basis, up to, and including, the morning dose on D5.

D2. Prior to the first dosing of the day, an instrumental measure of erythema was performed at one test site (R1p) and at one non-patched, control site (R1np) on the *right* upper back (paraspinal region) of each subject just prior to patch application. Three nickel sulfate patches were applied on the skin of the designated region (sites R1p, R2p, and R3p).

D3. The study medication was administered.

D4. The three nickel sulfate patches were removed approximately 48 hours after application. Visual clinical scores (R1p, R2p) and instrumental measures of erythema (R1p, R1np) were performed approximately 1 hour after the patch removal (48 + 1 hours after patch application). Under local lidocaine anaesthesia, a 4-mm skin biopsy was taken from the centre of sites R2p and R3p, respectively. Biopsies were used to quantify the cellular influx into the dermis by immunohistology.

Pharmacokinetic evaluations

Blood samples for measurement of CP-481715 plasma concentrations were taken on D4 at 0 hour (predose), and serially 0.5, 1, 2, 4, 6, and 8 hours after the first dosing. The samples were stored at –20°C until shipment for assay. CP-481715 concentrations in plasma were measured using a validated high performance liquid chromatography/tandem mass spectrometry (LS/MS/MS) method. Pharmacokinetic parameters were determined from plasma concentration–time data using standard non-compartmental methods.

D5 and D8 (72 and 144 hours after patch application). Clinical visual scorings (R1p) and instrumental measurements of erythema (R1p, R1np) were continued to be performed at the designated sites.

D12 to D15. Follow-up visit with removal of sutures from biopsy sites.

Immunohistology of skin biopsies

The cells expressing CD4 (T helper cells), CD8 (T suppressor cells), CD68 (monocytes/macrophages)

and CCR1⁺ cells were quantified from formalin-fixed, paraffin-embedded 5- μ m tissue sections. Monoclonal antibodies directed against CCR1 (R & D Systems, Minneapolis, MN), CD68, CD4, and CD8 (Dako, Carpinteria, CA, USA) were used at final dilutions that ranged from 1 to 10 μ l/ml following specific permeabilization steps as outlined by the manufacturers. Matched isotype immunoglobulins were used as negative controls and further staining steps were as noted in previous publications (14, 15). The number of immunoreactive cells per millimeter squared of dermis was determined by counting an area of 4×4 reticules, at $\times 400$ magnification using an Olympus BX60 microscope (Olympus, New Hyde Park, NY, USA) for all markers by a single blinded investigator.

Safety evaluations

Safety evaluations included continuous clinical monitoring and registration of adverse events (AEs), safety laboratory tests (predose and on D8), vital signs (predose, on D2, D5, and D8) and 12-lead electrocardiograms (screening).

Statistical methods

The sample size was calculated to provide 80% power to detect a 50% reduction in area under the curve (AUC) of instrumentally measured erythema, and of change from baseline CD68⁺ cell count, using a one-sided 5% significance level. Summary statistics were calculated for all pharmacokinetic parameters. All statistical tests comparing CP-481715 with placebo were performed at a one-sided 5% significance level. No adjustments were made for multiple comparisons. Data from all the study sites were analysed and covariate adjusted if needed. Safety data were evaluated using descriptive statistics.

Clinical visual scoring

The original clinical scores (0, $\pm$, +, ++, and +++) were converted to numerical scores (0, 0.5, 1, 2, and 3), *a priori*. For each subject and each site (L1p and R1p), an AUC value was calculated using trapezoid rules for the clinical scores obtained before and at 48 + 1, 72, and 144 hours after patch application. The AUC value was considered as a weighted average of the clinical scores over time. AUC (L1p) obtained at screening served as baseline value. The change in AUC measure from baseline to study period was the analysis variable.

Instrumental measurements of erythema

For the sites L1p, L1np, R1p, and R1np, AUC values were calculated as described above. The

following differences in AUC between patched and non-patched sites were calculated: $AUC(L) = AUC(L1p) - AUC(L1np)$ and $AUC(R) = AUC(R1p) - AUC(R1np)$. AUC(L) served as baseline value. The change from baseline was calculated as $[AUC(R) - AUC(L)]$ and used as the analysis variable.

Skin biopsy measures

For the CD4⁺, CD8⁺, CD68⁺, and CCR1⁺ cell counts obtained from skin biopsies taken 48 + 1 hours after nickel patch application, the counts from site L2p obtained at screening were considered the baseline values. The change from baseline value was the analysis variable.

To assess treatment effects, each of the CP-481715 groups was compared with pooled placebo in terms of change from baseline, using a fixed effects model with fixed terms for treatment and investigative sites. For comparisons between CP-481715 regimens versus pooled placebo, the mean difference in change from baseline was reported with 90% confidence limits and the *P* value for the treatment difference. The SAS[®] software program version 8.2 (SAS Institute Inc., Cary, NC, USA) was used to perform the data summarization and statistical analyses.

Results

Subject disposition and demography

A total of 40 subjects were randomized: 13 subjects received CP-481715 1000 mg TID, 12 subjects CP-481715 3000 mg QD, and there were 15 subjects in the placebo groups (placebo TID: *n* = 8; placebo QD: *n* = 7). No subject withdrew from the study. Subjects included 13 male and 27 female subjects who ranged in age from 21 to 60 years. The subjects were predominantly white (33 subjects). The demographic characteristics are presented in Table 1.

One subject, who was randomized to placebo, received CP-481715 1000 mg TID by mistake, and was not included in the pharmacokinetic analyses; this subject was included in the placebo TID group for efficacy and safety analyses.

Clinical visual scores

Pretreatment with 1000 mg CP-481715 TID resulted in significant, clinical reductions in the visual scores of the nickel patch test reactions as the mean change from baseline $[AUC(R1p) - AUC(L1p)]$ for the CP-481715 1000 mg TID group was significantly lower than the mean change from baseline for the pooled placebo group [Difference: -50.13 (90% Confidence

Table 1. Demographic characteristics: mean (SD) values^a

No. subjects	CP-481 715 1000 mg TID (n = 13)	CP-481 715 3000 mg QD (n = 12)	Placebo TID (n = 8)	Placebo QD (n = 7)
Age (years)				
Male	33.3 (9.6)	38.0 (15.0)	24.0 (0.0)	32.5 (12.0)
Female	50.9 (9.8)	49.6 (5.6)	45.3 (15.9)	48.8 (10.0)
Weight (kg)				
Male	78.2 (11.5)	81.8 (8.7)	81.0 (0.0)	80.0 (31.1)
Female	77.9 (18.5)	69.0 (7.3)	80.0 (31.1)	68.2 (7.8)
BMI (kg/m ²)				
Male	25.2 (2.7)	24.6 (3.3)	25.0 (0.0)	23.9 (7.0)
Female	29.6 (5.8)	25.6 (2.7)	27.5 (10.6)	24.9 (2.2)

BMI, body mass index; QD, once daily; SD, standard deviation; TID, three times daily.

^aIn order to recruit subjects it was necessary to enroll subjects up to 60 years of age or a BMI > 35 kg/m². These protocol deviations did not affect subject safety or the interpretation of the study results.

Interval (CI) –82.17, –18.09), $P = 0.01$]. None of the subjects experienced total inhibition of the allergic reactions to nickel. No significant change from pooled placebo was observed for the CP-481715 3000 mg QD group ($P = 0.80$). A summary of the clinical visual scores and AUC values by treatment group is provided in Table 2.

Instrumental measurements of erythema

Similarly, pretreatment with 1000 mg CP-481715 TID also showed signs of reducing erythema reactions to nickel when assessed with the chromameter. The mean change from baseline [AUC(R) – AUC(L)] for the 1000 mg CP-481715 TID group tended to be lower than the mean change from baseline for the pooled placebo group [Difference: –186.97 (95% CI: –349.01, –24.92), $P = 0.06$]. No significant change from pooled placebo was observed for the 3000 mg CP-481715 QD group ($P = 0.13$). A summary of the measurements of erythema and AUC values by treatment group is provided in Table 3.

Immunohistological cell counts

Cell counts for CD4⁺, CD68⁺, and CD8⁺ cells increased significantly between non-patched predose and patched baseline biopsy samples ($P < 0.01$), while CCR1⁺ cell counts decreased ($P = 0.02$). There were no significant differences observed between either CP-481715 treatment group and pooled placebo for any of the immunohistological end-points ($P \geq 0.13$). However, whereas the numbers of inflammatory cells tended to increase in biopsies after the second nickel challenge in the placebo groups, the cell counts tended to remain stable or even decrease for the CP-481715 1000 mg TID group, particularly for CD4⁺ and CD8⁺ cells.

Pharmacokinetics of CP-481715

Following multiple-dose administration to healthy subjects, exposures of CP-481715 were highly variable between subjects following both dosing regimens. The mean CP-481715 concentrations at D4 are presented in Fig. 1 by treatment

Table 2. Summary of clinical visual scores and AUC values by treatment group: mean (SD) values^a

Parameter	CP-481-715 1000 mg TID (n = 13)	CP-481-715 3000 mg QD (n = 12)	Pooled placebo (n = 15)
Screening: L1p site			
AUC	252.2 (49.16)	238.5 (62.21)	240.1 (50.34)
Clinical visual scores			
49 hours	2.1 (0.28)	2.1 (0.29)	2.0 (0.00)
72 hours	2.3 (0.48)	2.1 (0.51)	2.2 (0.41)
144 hours	1.9 (0.74)	1.8 (1.05)	1.8 (1.01)
Study period: R1p site			
AUC	177.8 (75.17)	220.5 (66.56)	217.4 (54.21)
Clinical visual scores			
49 hours	1.7 (0.69)	2.0 (0.60)	1.9 (0.26)
72 hours	1.6 (0.65)	2.1 (0.67)	2.0 (0.65)
144 hours	1.2 (0.77)	1.4 (0.57)	1.5 (0.61)
Change from baseline			
AUC(R1p) – AUC(L1p)	–74.4 (53.55)	–18.0 (39.29)	–22.7 (49.24)

AUC, area under the curve; QD, once daily; SD, standard deviation; TID, three times daily.

^aBaseline was screening value.

Table 3. Summary of instrumental measurement of erythema values by treatment group: mean (SD) values^a

Parameter	CP-481-715 1000 mg TID (n = 13)	CP-481-715 3000 mg QD (n = 12)	Pooled placebo (n = 15)
Screening: L1p site			
AUC(L1p)	2440 (334.2)	2832 (1432)	2399 (403.6)
AUC(L) = AUC(L1p) – AUC(L1np)	1130 (281.5)	877.0 (587.1)	971.5 (397.4)
Erythema			
0 hours	8.9 (2.29)	12.9 (12.73)	9.6 (1.95)
49 hours	17.7 (3.28)	20.5 (9.97)	17.1 (2.99)
72 hours	19.3 (3.46)	21.3 (9.68)	18.8 (2.96)
144 hours	18.3 (2.69)	21.3 (9.47)	18.5 (4.72)
Study period: R1p site			
AUC(R1p)	2110 (421.7)	2584 (1490)	2318 (309.1)
AUC(R) = AUC(R1p) – AUC(R1np)	873.4 (383.4)	764.0 (538.2)	967.3 (333.2)
Erythema			
0 hours	8.0 (1.95)	12.3 (13.66)	9.5 (2.21)
49 hours	16.2 (3.95)	18.1 (11.74)	17.0 (3.12)
72 hours	16.6 (3.84)	19.9 (9.33)	18.0 (2.71)
144 hours	15.0 (3.30)	19.0 (8.98)	17.1 (2.90)
Change from baseline [AUC(R) – AUC(L)]	–330 (435.9)	–248 (321.3)	–80.3 (287.0)

AUC, area under the curve; QD, once daily; SD, standard deviation; TID, three times daily.

^aBaseline was AUC(L) value.

arm. As expected, CP-481715 mean plasma concentrations over the 8-hour sampling period were generally higher for the 3000 mg QD dosing than for the 1000 mg TID dosing.

Table 4 presents mean PK parameters for CP-481715 following multiple administrations of CP-481715 dosed TID or QD.

Safety results

Any AE occurring following the start of treatment was considered as treatment emergent. A total of 81 treatment-emergent AEs were reported by 29 of the 40 subjects and 63% of these AEs were considered treatment related (51/81) by the investigator. A total of 72% (18/25) of the subjects

receiving CP-481715 and 73% (11/15) of the subjects receiving placebo experienced an AE. The incidence of AEs was similar between subjects who received CP-481715 1000 mg TID versus CP-481715 3000 mg QD (69% versus 75%, respectively). Most AEs were related to the body as a whole and the digestive body system. The most frequently reported AEs included asthenia (10 subjects), headache, and flatulence (seven subjects each), diarrhoea, and abdominal pain (four subjects each). Most AEs were mild in severity; only 28% (23/81) of the AEs were considered moderate in intensity. There were no serious adverse events (SAEs). There were no discontinuations because of the AEs reported during this study. No subjects had changes in vital signs or laboratory tests of clinical significance.

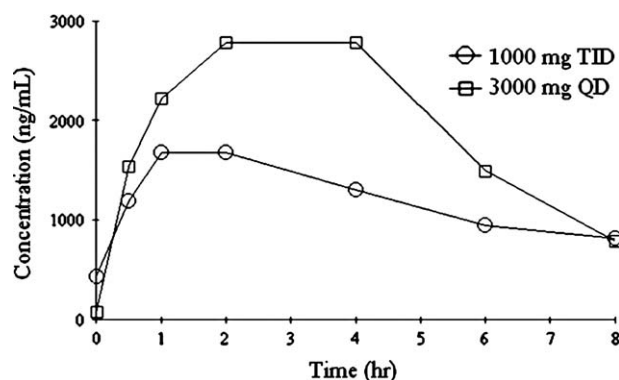


Fig. 1. Mean concentration–time profiles after multiple oral doses of CP-481 715 Oral Powder for Constitution to Healthy Adult Subjects. The mean CP-481 715 concentrations at D4 are presented by treatment arm in this figure. $n = 12$ for both treatments, except at sixth and eighth hours where $n = 5$ for both treatments. hr, hours; QD, every day; TID, three times daily.

Table 4. Mean (SD) CP-481 715 pharmacokinetic parameters following the administration of multiple oral doses to healthy adult subjects^a

Pharmacokinetic parameters (units)	1000 mg TID (n = 12)	3000 mg QD (n = 12)
AUC ₄ (h • ng/mL) ^b	5240 (2240)	8670 (4030)
C _{max} (ng/mL) ^b	1800 (762)	2970 (1390)
C _{min} (ng/mL) ^b	382 (232)	67 (28) ^c
T _{max} (h) ^d	2 (1–8)	2 (1–4)

AUC₄, area under the plasma concentration–time curve from time zero to time 4 hours; C_{max}, maximum observed concentration; C_{min}, minimum observed concentration; QD, once daily; SD, standard deviation; TID, three times daily; T_{max}, time to maximum plasma concentration.

^a $n = 12$ for both treatments.

^bGeometric mean.

^c $n = 11$.

^dMedian (range).

Discussion

Some animal disease models have shown that inhibition of the CCR1 receptor or its ligands abrogates disease. The present study was conducted to assess the effect of the CCR1 receptor antagonist, CP-481715 on clinical and cellular responses in ACD in humans.

At the screening, the nickel challenge induced the expected clinical reactions and induced marked increases in cellular responses as measured by cell infiltration in skin biopsies. During the study period, the clinical reactions to the nickel challenge were significantly reduced by pretreatment with 1000 mg CP-481715 mg TID, suggesting that the CCR1 pathway may be involved in ACD in humans: As measured by AUC-values, there were statistically significant decreases in mean clinical visual scores from baseline to the study period for subjects receiving 1000 mg CP-481715 TID versus the pooled placebo group.

The instrumentally measured erythema values tended to decrease from baseline to the study period for subjects in the 1000 mg CP-481715 TID group compared with the pooled placebo group. No differences in clinical visual scores or instrumental measurements of erythema were noted between the 3000 mg CP-481715 QD group and pooled placebo.

There were no significant differences observed between either CP-481715 treatment group and pooled placebo for any of the immunohistological end-points (CCR1⁺, CD4⁺, CD68⁺, and CD8⁺ cell counts). However, consistent with the clinical reactions to nickel that faded but were not totally inhibited, the cell infiltration data for the 1000 mg CP-481715 TID group did show a small numerical reduction in the number of CD4⁺ and CD8⁺ cells during the study period compared with the baseline challenge, while there was a small increase in cell counts in the placebo group (data not shown). Nevertheless, a high variability was observed in the cell infiltration data, so these possible trends should be interpreted with caution. The high variability suggests that this study might have been underpowered for this outcome variable.

The 1000 mg CP-481715 TID dosage regimen proved to be more efficacious than the 3000 mg CP-481715 QD regimen, even though the total daily dose was equal in the two groups. Despite higher exposure (AUC and $C_{\max}$) during the first 4 hours after administration of a single 3000 mg CP-481715 dose, the 3000 mg CP-481715 QD dosage regimen was associated with lower predose trough concentrations ($C_{\min}$) than the 1000 mg CP-481715 TID regimen (Table 4), suggesting that the

inhibitory effects of CP-481715 on the immune response may require a sustained level of drug above a certain minimum concentration, corresponding to a minimum inhibitory concentration.

CP-481715 administered at doses of 3000 mg QD or 1000 mg TID for 5 days was generally considered safe and well tolerated in these healthy but nickel-allergic subjects. There were no SAEs or discontinuations because of AEs and most treatment-related AEs were of mild severity. A considerable part of the treatment-related AEs consisted of gastrointestinal discomfort that was potentially related to the bitter and unpleasant taste of the dosing formulation.

Treatment with CP-481715 reduced, but did not prevent clinical reactions to nickel in the present study. There are several possible explanations for the insufficient clinical efficacy. It is possible that the dose of CP-481715 used in these healthy but allergic subjects was too low, or that the induced allergic reactions were too limited to detect significant changes. Furthermore, the timing of the dosing of CP-481715 in relation to the nickel challenge might not have been ideal; it is possible that several days of pretreatment with CP-481715 might be necessary for sufficient inhibition of the inflammatory response in humans. However, the most likely explanation for the lacking success of the treatment relates to the complex nature of the chemokine network, which is activated during inflammatory skin diseases (16). Goebeler et al. (7) analysed the pattern of chemokine expression in the skin of ACD subjects at different time-points after hapten application and showed that CCL2/MCP-1 is the first chemokine appearing in the basal epidermal layer as early as six hours after allergen contact thus clearly preceding infiltration by monocytes and lymphocytes. At 12 hours epidermal expression of MCP-1 was increased and was accompanied by epidermal, and to a lesser extent, dermal expression of CCL5/RANTES, and exclusive dermal expression of CCL22/macrophage-derived chemokine. At 24 hours, these chemokines were accompanied by epidermal expression of IP-10 and monokine induced by interferon- γ (MIG) and dermal expression of CCL17/thymus- and activation- regulated chemokine and CCL18/pulmonary and activation-regulated chemokine, coinciding with a strong infiltration of monocytes and lymphocytes. IP-10, MIG, and interferon-inducible T-cell alpha chemoattractant (I-TAC) are ligands of the chemokine receptor for CXC chemokine (CXCR)3. Another study has shown that nickel-specific CD4⁺ and CD8⁺ T-cell lines established from ACD skin produced IFN- γ and IL-4 and expressed moderate to high

levels of CXCR3 receptors. IL-4 exerts a pro-inflammatory stimulation on keratinocytes by potentiating the IFN- γ and TNF- α -induced production of IP-10, MIG, and I-TAC that in turn attract CXCR3⁺ T lymphocytes to the inflammatory reaction site (17). Thus, leucocyte recruitment during ACD is under the control of sequential and coordinated release of numerous chemokines from both activated resident cells in the skin as well as infiltrating leucocytes resulting in successive boosts of lymphocyte arrivals (7, 16). Other studies have demonstrated an upregulation of CCR10⁺ skin-homing T cells induced by CCL27/cutaneous T-cell attracting chemokine in nickel-exposed skin of ACD subjects (18). Furthermore, nickel-specific CD4⁺ and CD8⁺ T-cells from ACD subjects have been shown to display distinct patterns of chemokine receptor expression, including CXCR3, CCR4, and CCR5 according to the involved subset of T-cell lines (19). In conclusion, several chemokine receptors seemed involved in ACD. Individual infiltrating lymphocytes and monocytes bear more than one receptor and many of the chemokines detected in ACD bind to different receptors enabling a strong persistent stimulation of migration effector cells despite a partial unresponsiveness of one or two receptor pathways after ligand binding (7). Consequently, the therapeutic validity of specifically targeting only one or a few of the chemokine receptors has to be questioned. Presumably, several targets have to be inhibited in order to obtain clinical efficacy, not only in ACD, but also in other inflammatory (skin) diseases.

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