Synopsis

Study Title:

A randomized, single-blind, saline-controlled, dose-finding study to characterize the pruritus and inflammatory response to intradermal histamine in healthy volunteers and patients with atopic dermatitis

Brief Title:

Characterization of a pruritus challenge model

Study Number:

CHDR1907

Study Phase:

Phase 1b

Name of Investigational Product:

Cetirizine 2HCl 10 mg

Study Sponsor:

Maruho Co,Ltd
1-5-22 Nakatsu, Kita-ku
Osaka, Japan

Regulatory Agency Identifier Number:

EudraCT number: 2019-004261-40
Toetsing Online number: NL71946.056.19
Independent Ethics Committee number: 056

Principal Investigator, Number of Study Centre(s) and Countries:

This study was conducted at a single centre (CHDR, Leiden, The Netherlands) that enrolled participants in The Netherlands.

The Principal Investigator was Prof. Dr. R. (Robert) Rissmann.

Study Period:
21 January 2020 (signed informed consent by first participant) to 11 August 2021 (Last subject last visit).

Background and Rationale

In many dermatological diseases, pruritus (or itch) is one of the impactful and burdensome symptoms patients face every day. Although pruritus by itself is seen as a benign symptom, pruritus can have adverse effects on the patients' wellbeing and daily life. In addition, chronic

itch is often accompanied by several unpleasant sensations such as pain and/or burning sensation. The mechanisms that underlie pruritus are not well known and are compounded by the subjective nature of itch. The primary sensory nerve fibers that innervate the skin are categorized into three groups based on the degree of myelination, diameter, and conduction velocity. The thick myelinated Aβ fibers transmit tactile sensation, whereas the thinly myelinated Aδ and unmyelinated C-fibers are mainly involved in the conduction of thermal, pain and itch sensation. Itch is transmitted predominately by these unmyelinated, slow conducting C-fibers. These fibers extend to the dermo-epidermal junction with free endings penetrating into the epidermis where sensation is detected. The cell bodies for these fibers are in the dorsal root ganglia (DRG), just outside the spinal cord. From here, both sensations involve secondary transmission neurons that ascend via the contralateral spinothalamic tract to the thalamus. Pruritogens interact with receptors or ion channels on the nerve fibers. The receptors that are often involved are G-protein coupled receptors (GPCR). GPCRs detect and respond to a diverse range of ligands or stimuli and are the target of many drugs. GPCRs that are relevant to itch respond to histamine, prostaglandins, neuropeptides, and proteases. The ion channels that appear to be primarily involved are members of the transient receptor potential (TRP) family. As an example, TRPV1 detects capsaicin, the active ingredient in chili peppers. Various drugs with different mechanisms of action are currently in development. These drugs have the potential to lead to targeted therapy of peripheral itch independent of blocking inflammation. For clinical drug development efficient and effective itch provoking challenge models in humans are needed. For these purposes, a variety of different compounds including cowhage, capsaicin and histamine have been tested. Ample experience has been obtained with histamine in studies, which is also used as positive control in skin prick tests in clinical practice for allergy testing. However, hardly any bioquantitative measurements have been performed to characterize the itch response following histamine injection. Therefore, the aim of this study was to characterize the dose-pruritogenic response upon intradermal histamine injection in healthy volunteers and patients with atopic dermatitis. This setup created a challenge model that temporarily induces skin itch which enables future application as proof-of-pharmacology or drug profiling in drug developmental programs. With the administration of oral antihistamine, the reversal of a fixed histamine-dose effect was investigated.

Objectives and Endpoints

Objectives	**Endpoints**
Primary • To characterize the pruritus response on histamine dose • To profile the response to intradermal histamine	Patient reported outcomes - Visual Analogue Scale (VAS) pruritus Non-invasive measures - Perfusion by Laser Speckle Contrast Imaging (LSCI) - Erythema by multispectral camera (Antera) - Wheal and flare by clinical evaluation (erythema grading scale) - Wheal and flare by Antera 3D

	- Erythema by colorimetry (DSM III) • Invasive measures - Skin punch biopsies ○ ○ Readout measures may comprise, but are not limited to: Immunohistochemistry: - Eosinophils - Monocytes/macrophages - Mast cells - NaV 1.5, 1.7, 1.8 and 1.9 staining qPCR (exploratory): - NaV 1.5, 1.7 and 1.8 expression
Secondary • Comparison to itch induction in healthy volunteers and patients with atopic dermatitis	- Patient reported outcomes Visual Analogue Scale (VAS) pruritus
• To assess safety and tolerability of intradermal histamine challenge	- Treatment-emergent (serious) adverse events ((S)AEs), concomitant medication, clinical laboratory tests (haematology, chemistry and urinalysis), vital signs (pulse rate, systolic blood pressure, diastolic blood pressure, temperature) and ECG (HR, PR, QRS, QT and QTcF)

Methodology:

This was a randomized, single-blind, saline-controlled, dose-finding, double- dummy study to characterize the pruritus and inflammatory response to intradermally administered histamine dihydrochloride (2HCl) in various concentrations to healthy volunteers (all parts) and patients (part A and C) with atopic dermatitis (AD). The study was divided in three parts (A, B, C) of which the first two (A and B) were intended to find the right administration route as well as the right concentration of the pruritogenic agent histamine. In part C of the study, oral antihistamine (cetirizine) was given as a positive control to assess reversal of the histamine- induced pruritus challenge. Part A consisted of two visits on separate days. On the first day, two different concentrations of histamine 2HCl were administered by Dermojet on the volar forearms to assess pruritic perception. During the second visit the upper back was challenged with three concentrations of histamine 2HCl and saline administered by Dermojet for characterization of the skin response. Part B of the study had a cross-over design in which histamine 2HCl was administered by intradermal injection and skin prick to healthy volunteers. This part of the study

was divided in two visits, visit day 1 and visit day 3. Visit day 1 and 3 consisted of three or four rounds depending on the administration method and in each round the volar forearms were challenged with either histamine 2HCl or 0.9% sodium chloride (NaCl). Each round continued for approximately 60 minutes. After each performed challenge, a switch was made to the contralateral volar forearm, resulting in a total of maximum two treatments per arm. After part A and B, an interim analysis was performed. Based on the available data, skin prick was chosen as the preferred administration method and 1 mg/mL histamine 2HCl as the most suitable concentration for part C. Part C of the study also consisted of two visit days and two rounds per visit day, with identical order of assessments for both visits. One round consisted of the following assessments:

- Pre- treatment with oral placebo of cetirizine
- After 60 minutes, the volar forearms were challenged with histamine 2HCl or 0.9% NaCl. VAS itch was assessed for 30 minutes
- Subsequently after completion of VAS itch, the upper back was challenged twice (one target area was for observational purposes and one for biopsy) with the same agent as the forearms approximately 30 min later.

Number of Participants (Planned and Analysed):

	Number of Participants (Population)				
	Randomized (Planned)	**Randomized (Analysed)**	**Completed**	**Safety**	**PD**
Part A	18	13	10	13	13
Part B	8	8	8	8	8
Part C	16	16	16	16	16

Abbreviations: PD = pharmacodynamic

Diagnosis and Main Criteria for Inclusion and Exclusion:

Key inclusion criteria:

All participants

1. Fitzpatrick skin type I-II (Caucasian)
2. Able and willing to give written informed consent and to comply with the study restrictions

Healthy volunteers

3. Healthy male subjects, 18 to 45 years of age, inclusive. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, haematology, blood chemistry, blood serology and urinalysis

AD patients

4. Male and female subjects with mild to moderate AD 18 to 65 years of age, inclusive. The health status is verified by absence of evidence of any clinically significant active or uncontrolled chronic disease other than AD following a detailed medical history and a complete physical examination including vital signs, 12-lead ECG, haematology, blood chemistry, blood serology and urinalysis
5. Suitable target of the affected skin defined as an eczema lesion of at least 1% BSA for each lesion (volar forearms and/or preferably upper back, total 3 lesions)
6. VAS itch of ≤30 at screening and prior to first administration of target lesions

Key exclusion criteria:

All participants

1. Diseases associated with immune system impairment, including auto-immune diseases, HIV and transplantation patients
2. History of pathological scar formation (keloid, hypertrophic scar)
3. Use of antihistamines within 3 weeks prior to start of the study
4. Subjects who show skin reaction to Skin marker

Healthy volunteers

5. Subjects suffering from chronic itch defined as presence of pruritic symptoms lasting more than 6 weeks
6. Have known history of atopy

AD patients

7. Requirement of immunosuppressive or immunomodulatory medication within 30 days prior to enrolment or planned to use during the course of the study

Study Treatments, Dose, Mode of Administration, and Batch Number(s):

Investigational Product:

Histamine dihydrochloride was used in this study as a challenge agent in different concentrations and dosing administrations as follows:

Treatment, dose, mode of administration				
	Treatment	**Concentration**	**Anatomical location**	**Mode of administration**
Part A	histamine 2HCl	500 µg in 0.1 * mL NaCl 1000 µg in 0.1 mL NaCl 2000 µg in 0.1 mL NaCl	Volar forearms and upper back	Dermojet
		1 µg in 0.1 mL NaCl 10 µg in 0.1 mL NaCl 100 µg in 0.1 mL NaCl	Volar forearms and upper back	Dermojet
Part B	histamine 2HCl	1 µg in 0.1 mL NaCl 10 µg in 0.1 mL NaCl 100 µg in 0.1 mL NaCl	Volar forearms	Intradermal injection
		1 mg/mL 10 mg/mL	Volar forearms	Skin prick
Part C	histamine 2HCl	1 mg/mL	Volar forearms and upper back	Skin prick

**The concentrations were revised after dosing the first three subjects.*

Control Product:

0.9%NaCl was administered as negative control in all parts of the study. The mode of administration was different between parts and within part B: Dermojet, intradermal injection or skin prick.

In part C of the study, cetirizine 2HCl 10 mg served as positive control to reverse clinical manifestations after challenge.

Duration of Study Treatment:

In part A and B the treatment period with histamine was divided in two days: on visit day 1 the forearms were challenged in four rounds with an interval of approximately 60 minutes. In these rounds either histamine 2HCl 1 µg, 10 µg or 100 µg or 0.9% NaCl were administered. On visit day 2 (part A), the same dosing sequence was administered consecutively on the upper back of healthy volunteers and lesional sites of AD patients for imaging purposes. In AD patients without an eczematous lesion on the upper back, another affected site was selected. In part B of the study the visits were separated by 2 days (visit day 1 and visit day 3) and histamine 2HCl or NaCl was

administered by intradermal injection and skin prick in a cross-over way. Part C of the study was divided in two days on which pre-treatment with 10 mg of cetirizine or placebo and afterwards 1 mg/ml histamine 2 HCl or saline were dosed on both days.

On visit day 1 and visit day 3, one forearm and the upper back were challenged. Two suitable target spots on the upper back (HV) and lesional sites (AD patients) were chosen for the challenge followed by invasive and non-invasive assessments. Cetirizine 2HCl 10 mg was orally administered once on both days, 60 minutes prior to the challenge on volar forearms.

Statistical Methods:

All pharmacodynamic endpoints were (1) listed by subject, treatment, group, body part (arm or back) and time, and individual plots by treatment and time were generated; (2) summarized by treatment and time; and (3) were analysed with a mixed model analysis of variance with fixed factors treatment, time and treatment by time, random factors subject, subject by treatment and subject by time. The following contrasts are calculated within the models:

Histamine 1 ug vs Placebo

Histamine 10 ug vs Placebo

Histamine 100 ug vs Placebo

Inferential analysis per endpoint was generated with estimates of the difference of the different contrasts and a back transformed estimate of the difference in percentage for log transformed parameters, 95% confidence intervals (in percentage for log-transformed parameters) and Least Square Means (geometric means for log transformed parameters), and the p-value of the contrasts. Least Square Means graphs are generated, with the Least Square Means of the analysis, and, if applicable, with the Least Square Means of the analysis of the data as change from baseline.

All calculations were performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

Summary of Results and Conclusions:

Participant Disposition

In part A 40 participants were screened, and 18 participants were enrolled. Treatment compliance was 100%. In total, 10 participants completed the study.

In part B 13 participants were screened, and 8 participants were enrolled. Treatment compliance was 100%. In total, 8 participants completed the study.

In part C 16 participants were screened, and 16 participants were enrolled, 8 of them participated in part B as well. Treatment compliance was 100%. In total, 16 participants completed the study.

Demographic and Other Baseline Characteristics:

No meaningful differences were notes between parts.

Characteristic	Category/ Statistics	Part A (N = 13)	Part B (N = 8)	Part C (N=16)
Sex	Female	0 (0%)	0 (0%)	3
	Male	13 (100%)	8 (100%)	13
Race	Asian	0 (0%)	0 (0%)	0 (0%)
	Black	0 (0%)	0 (0%)	0 (0%)
	Other	0 (0%)	0 (0%)	0 (0%)
	White	13 (100%)	8 (100%)	16 (100%)
Age (y)	n	13	8	16
	Mean (SD)	23.4 (4.6)	21.6 (2.3)	24.3 (5.7)
Weight (kg)	n	13	8	16
	Mean (SD)	78.0 (9.7)	76.1 (8.3)	73.5 (9.5)
Height (cm)	n	13	8	16
	Mean (SD)	184.6 (9.7)	185.0 (5.1)	180.3 (7.8)
BMI (kg/m^2)	n	13	8	16
	Mean (SD)	23.0 (2.8)	22.3 (2.3)	22.6 (2.2)

Exposure:

Initially in part A the study participants were exposed to 500µg, 1000µg and 2000µg in 0.1 mL NaCl and 0.9%NaCl, resulting in a total exposure of 3500µg histamine 2HCl.

In part A of the study participants were exposed to different concentrations of histamine: 1 µg histamine 2HCl in 0.1 mL NaCl, 10 µg in 0.1 mL NaCl and 100 µg in 0.1 mL NaCl. This resulted in a total exposure of 222 µg histamine 2HCl.

In part B, study participants were exposed to 1 µg histamine 2HCl in 0.1 mL NaCl, 10 µg in 0.1 mL NaCl, 100 µg in 0.1 mL NaCl and 0.9% NaCl by intradermal injection and histamine 2 HCl 1mg/mL, 10mg/mL and 0.9% NaCl by skin prick. The total exposure for intradermal injection was 111 µg histamine 2HCl. For skin prick testing it is more difficult to calculate the total exposure since the solution diffuses into the skin and therefore the exact dosing is unknown.

In part C, healthy volunteers and patients with AD were exposed to 10 mg cetirizine 2HCl and placebo followed by 1 mg/mL histamine 2HCl (skin prick) to the volar forearms and the upper back or lesional area. The total exposure of histamine 2HCl could not be calculated, for cetirizine 2HCl total exposure was 20 mg.

Safety Results:

In part A of the study, four treatment emergent adverse events occurred, two of them being administration site urticaria, one injection site haematoma and one injection site pain. One treatment emergent adverse event was observed in part B of the study that was categorized as

gastrointestinal disorder as nausea was reported. In part C of the study, no treatment emergent adverse events were observed.

	Number (%) of Participants		
	Part A (N = 13)	**Part B (N = 8)**	**Part C (N=16)**
All AEs	4 (30.8%)	1 (12.5%)	0 (0%)
Treatment-related AEs	4 (30.8%)	1 (12.5%)	0 (0%)
Severe AEs	0 (0%)	0 (0%)	0 (0%)
AEs leading to death	0 (0%)	0 (0%)	0 (0%)
SAEs	0 (0%)	0 (0%)	0 (0%)
Treatment-related SAEs	0 (0%)	0 (0%)	0 (0%)
AEs leading to discontinuation of study treatment	2 (15.4%)	0 (0%)	0 (0%)
AEs leading to discontinuation from study	2 (15.4%)	0 (0%)	0 (0%)

Pharmacodynamic Results:

The results below are described per part.

In part A, no clear pruritic response was observed following histamine 2HCl injection in all concentrations with Dermojet. The peak (SD) VAS itch of healthy volunteers was 6.3 (± 4.77), 10.4 (± 9.96) and 7.4 (± 11.19) for 1µg, 10µg and 100µg histamine 2HCl, respectively. For AD patients the maximum observed mean (SD) VAS itch was 16.5 (± 10.61), 5.5 (± 2.83) and 14.5 (± 7.78) for 1µg, 10µg and 100µg histamine 2HCl, respectively. Although no clear pruritic responses were seen, a clear dose dependent result was observed in the healthy volunteers' group for wheal and flare and erythema by means of 2D photography, multispectral imaging and perfusion.

In part B of the study VAS itch was assessed in 8 healthy male volunteers. For VAS itch the highest mean (SD) pruritic response was observed for skin prick 1 mg/mL 34.9 (± 25.48) followed by skin prick 10 mg/mL 29.8 (±22.80), Figure 2. Challenges with histamine 2 HCl administered by intradermal injection did not reach the scratching threshold. Subjects experienced minimal pain sensation with a VAS pain <10 mm for intradermal injection with placebo and skin prick with placebo. Similarly for skin prick with 1 mg/mL and 10 mg/mL histamine 2 HCl. For intradermal injection the VAS pain did not exceed 26 mm for 1µg, 10µg and 100µg histamine 2HCl.

Based on the results of part A and B, a pruritus model using 1mg/ml histamine 2HCl for administration by skin prick test was found to be the most suitable model.

In part C, the peak (SD) VAS itch was 26.4 (± 28.06) for the subjects that received pre-treatment with cetirizine before histamine 2HCl administration, and 17.8 (± 22.73) for the subjects that received pre-treatment with placebo before histamine 2HCl administration. There was not enough evidence to show a significant LSM difference (in %) in VAS itch induction between the contrast pre-treatment cetirizine + histamine and pre-treatment placebo + histamine HV (LSM difference = -8.4%, 95% CI: -49.4% - 66.0%, p=0.7683).

In addition, pre-treatment with cetirizine before histamine 2HCl administration had no effect on suppression of the skin perfusion between pre- treatment placebo + histamine and pre-treatment cetirizine + histamine (LSM difference=-16.011, 95% CI: -49.591 – 17.568, p= 0.3412) and did not result in a decrease of LSM perfusion (LSM difference=7.159, 95% CI: -26.509 – 40.827, p= 0.6699) caused by histamine challenge in AD patients. Furthermore, there was not enough evidence to show any difference between both populations, healthy volunteers, and AD patients.

Also, no inhibitory effects of pre-treatment with cetirizine were seen on erythema assessments in both healthy volunteers and patients with atopic dermatitis. Erythema did not differ between populations (HV and AD patients) LSM difference= -2.4362, 95% CI: -5.0187 – 0.1462, p= 0.0629.

Conclusions (all parts):

- Injection with histamine 2HCl via Dermojet did not lead to induction of itch but did lead to a dose-dependent induction of wheal and flare.
- Intradermal injection with histamine 2HCl did not induce itch above the scratching threshold, most likely due to pain sensation overruling the itch perception.
- Administration with 1 mg/mL histamine by skin prick did induce itch sensations above the pruritic threshold, and it was therefore concluded to be the most convenient administration method and dose for this challenge model.
- Pre-treatment with a single dose of 10 mg cetirizine 60 min before administration of 1mg/ml histamine 2HCl by skin prick did not suppress itch intensity over the tested timespan indicating that the effect of the antihistamine in tissue might occur later than the reported Tmax.
- Pre-treatment with a single dose of 10 mg cetirizine 60 min before administration of 1mg/ml histamine 2HCl by skin prick also did not suppress signs of wheal and flare (perfusion and erythema) over the tested timespan
- The current study design did not confirm reproducibility of the histamine skin prick model.
- Future studies should focus on reproducibility of histamine as challenge agent in models required for early phase clinical development.

Date and Version of this Report:

Version 1, dated 07 Nov 2022