Synopsis

Study Title:

A dermal inflammatory challenge study to evaluate complement activation in healthy volunteers.

Brief Title:

Local complement activation after dermal inflammatory challenge

Study Number:

CHDR2036

Study Phase:

0

Study Funder:

Q32 Bio

Regulatory Agency Identifier Number:

EudraCT number:

2020-005595-35

Toetsing Online number:

NL76227.056.20

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 Matthijs Moerland, PhD

Study Period:

19 February 2021 signed informed consent by first participant to 01 April 2021 last participant last visit

Background and Rationale

Inflammation is a response to damaged tissue and/or pathogens resulting in cellular activation and a release of cytokines. Although inflammation is in principle a healthy process, in some cases an excessive and/or poorly regulated inflammatory response can be harmful to the host, which is the case in many inflammatory disorders. Toll-like receptors belong to the family of pattern recognition receptors (PRRs). These highly conserved receptors recognize pathogen-associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). Detection of PAMPs by mediators of innate immunity brings multiple components of immunity into play, including the complement system. As such, the complement system is a first line of defense for fighting pathogens and clearing apoptotic cells. However, when

hyperactivated, it is a driver of a variety of autoimmune and inflammatory diseases. Investigational products are under development for regulation of complement, preferably directly to diseased tissues without long-term systemic blockade, minimizing the risk of serious infections and other complications. An in vivo complement activation model would be of great benefit for the early clinical evaluation of the pharmacological activity of novel complement-targeting investigational compounds, but such a model is not readily available. The study evaluated the capacity of 2 common and clinically well-characterized innate immune triggers (UV-B and imiquimod) to drive complement activation *in vivo*. Imiquimod is an imidazoquinolone drug acting as TLR7 agonist, exhibiting tumoricidal and anti-viral effects both *in vitro* and *in vivo* (Hanna, Abadi, and Abbas 2016). Aldara® (imiquimod 5%) cream is on the market for treatment of (pre)malignant and HPV-induced skin lesions (see SPC Aldara). CHDR has extensive experience with the topical imiquimod challenge model in which repeated exposure of tape-stripped skin to Aldara results in the development of psoriasis-like inflammatory lesions. The UV-B "sun burn" model is an inflammatory pain model in which erythema is induced on the skin by radiating the skin with UV-B light in a well-controlled and reproducible manner. UV-B exposure drives an increase in skin perfusion, followed by infiltration of immune cells increase into the skin. CHDR has applied this model frequently in the field of inflammatory pain studies. In this study, we aimed to evaluate complement activation after local imiquimod and UV-B exposure in healthy volunteers. Readouts were based on non-invasive measures (local erythema, perfusion, temperature) and invasive measures (IHC and mRNA analysis of skin punch biopsies for complement factors).

Objectives and Endpoints

Table 1: Objectives and endpoints

Objectives	Endpoints
Primary	
• To evaluate complement activation in skin after topical imiquimod challenge • To evaluate complement activation in skin after local UV-B challenge	• Complement factors in skin biopsies following imiquimod challenge • Complement factors in skin biopsies following UV-B challenge
Secondary	
• To characterize the clinical response to an UV-B and imiquimod challenge	• Perfusion by LSCI • Erythema by Antera 3D and clinical evaluation
Exploratory	
• To explore immune activation following UV-B challenge • To evaluate the correlation between complement activation and clinical response	• Cytokines or immune cells in skin biopsies • Perfusion by LSCI • Erythema by Antera 3D and clinical evaluation

Methodology:

This was a single-centre, two-part inflammatory challenge study in healthy volunteers, to evaluate complement activation by imiquimod and UV-B in two parallel groups of healthy volunteers. In the first study part, two cohorts of 5 volunteers underwent a topical UV-B or imiquimod challenge, accompanied by non-invasive imaging and serial biopsies of the challenge sites. The second part of the study was cancelled.

Part 1: UV-B challenge (n=5)

Screening: 1 hour admission

Study day (day 1): 7 hours admission (baseline biopsy, challenge, and 6hr biopsy)

Follow-up visit 1 (day 2): 1 hour admission (24hr biopsy)

Follow-up visit 2 (day 3): 1 hour admission (48hr biopsy)

Follow-up visit 3 (day 4): 1 hour admission (72hr biopsy)

End of study visit (+7 days): 1 hour admission

Part 1: Imiquimod challenge (n=5):

Screening: 1 hour admission

Study day (day 1): 2 hours admission (baseline biopsy and first challenge)

Follow-up visit 1 (day 2): 2 hour admission (24hr biopsy and second challenge)

Follow-up visit 2 (day 3): 1 hour admission (48hr biopsy and third challenge)

Follow-up visit 3 (day 4): 1 hour admission (72hr biopsy)

Follow-up visit 4 (day 6): 1 hour admission (120hr biopsy)

End of study visit (+7 days): 1 hour admission

Number of Participants (Planned and Analysed):

Number of Participants (Population)				
Randomized (Planned)	**Randomized (Analysed)**	**Completed**	**Safety**	**PD**
10	11	10	11	11

Abbreviations: PD = pharmacodynamic

Diagnosis and Main Criteria for Inclusion and Exclusion:

A total of 10 healthy male participants, 18 to 65 years of age were included. Main inclusion criteria included a Body mass index (BMI) between 18 and 30 kg/m^2, a minimum weight of 50 kg and Fitzpatrick skin type I-III (Caucasian). Main exclusion criteria included history of pathological scar formation (keloid, hypertrophic scar) or keloids or surgical scars in the target treatment area that in the opinion of the investigator, would limit or interfere with dosing and/or measurement in the trial; history of skin cancer (basal cell carcinoma,

squamous cell carcinoma, melanoma); any current and / or recurrent clinically significant skin condition at the treatment area (i.e. atopic dermatitis); including tattoos; history or presence of post-inflammatory hyperpigmentation and a minimal erythema dose (MED) higher than 355 mJ/cm2 at screening (applicable for the participants in the UVB-MITT population only).

Study Treatments, Dose, Mode of Administration:

Imiquimod
Aldara 5% is a cream containing the active ingredient imiquimod (50 mg/g). In general use, maximum application duration is up to 16 weeks with 3-5 applications per week depending on the indication (see SPC).

UV-B
As part of the screening assessments, the participant's Fitzpatrick skin photo type was determined (type I – VI). The participant was first exposed to 6 different doses of UV-B, to determine the Minimal Erythemic Dose (MED) expressed in J/cm^2, using the six different slots of the UV-B lamp. Twenty-four hours (± 2 hours) after the exposure of the 6 doses, the erythemic response of the skin to UV-B was assessed by two observers. The MED was determined visually, by observing which dose produces the first clearly discernible erythema.

Duration of Study Treatment:

Imiquimod
A dosage of 5 mg imiquimod (100 mg Aldara®) per treatment site was applied, for 3 days.

UV-B
On the treatment days, the participant's skin was exposed to two minimal erythema doses (2MED) of UV-B.

Statistical Methods:

No statistical testing was conducted. Data listings and averages were presented for challenge response and safety endpoints. Given the exploratory character of the study, challenge response were primarily analysed using descriptive statistics. All challenge response endpoints were summarized with at least mean and standard deviation of the mean, median, minimum and maximum values, by treatment and time, and were also presented graphically as mean over time, with standard deviation as error bars. All categorical efficacy endpoints were summarised by frequencies.

Summary of Results and Conclusions:

Participant Disposition

31 participants were screened, and 11 participants were enrolled. Treatment compliance was 100%. In total, 10 participants completed the study. One participant was discontinued because he had been in close contact with someone who was tested positive for COVID-19.

Demographic and Other Baseline Characteristics:

Participants had a mean age of 32.7 years (range: 19 to 55), 100% were male, and 100% were white. 9.1% of participants had Fitzpatrick skin type 1, 63.6% type 2 and 27.3% had

Fitzpatrick skin type 3. Demographics and other baseline characteristics were generally similar among treatment arms.

Exposure to non-investigational drug Imiquimod/ UV-B:

All 5 participants enrolled into the imiquimod group received a 100 mg dose of topical Imiquimod during three consecutive days. All cream weights were within 5% of the set dose of 100 mg. All 6 participants enrolled in the UV-B group received a dose of 2xMED. The MED was assessed during the screening visit. The average applied dose was 603.67 mH/cm^2.

Safety Results:

No SAEs, drug discontinuation due to AEs or deaths occurred during the conduction of this study. One mild treatment emergent adverse event was reported during the study period, namely application site pruritus. Other reported AEs were classified as unrelated to study treatment. The results from the current study show that dermal application of Imiquimod or UV-B radiation to induce and inflammatory response is safe and well tolerated in male healthy participants.

Pharmacokinetic Results:

Not applicable

Pharmacodynamic Results:

Visual assessment of erythema showed that Imiquimod challenge induced a more moderate response with increasing erythema after multiple applications (72h) whereas UV-B showed an acute response at 6h, which gradually decreased. Laser speckle analysis showed that Imiquimod challenge induces a buildable increase in basal flow with a higher peak value (mean 113.8 AU, SD 76.8 AU for area 4 at 72h) whereas UV-B challenge induces a quick response that peaks after 6h (mean 82.1 AU, SD 41.7 AU for area 4) and gradually declines. Erythema analysis (measured with Antera 3D camera) showed that Imiquimod challenge induces a buildable, slower onset increase in erythema compared to UV-B, with a slightly higher peak value in the imiquimod challenge (mean 15.4 AU, SD 3.9AU at 6h for target area 4 UV-B and mean 17.5 AU, SD 7.5 at 72h for target area 4 imiquimod). In naïve, unchallenged skin, trace or mild C3d staining was observed in the dermis, with no significant C3c deposition. No significant induction of C3c was seen at any time point after imiquimod or UV-B challenge.

Efficacy Results:

Not applicable

Conclusions:

The results from the current study show that dermal application of Imiquimod or UV-B radiation to induce and inflammatory response is safe and well tolerated in male healthy participants.Visual assessment of erythema as well as Imaging via Laser Speckle analysis and Antera 3D camera showed that Imiquimod challenge induces a more moderate, buildable response with increasing erythema and basal flow after multiple applications (72h) whereas UV-B showed an acute response at 6h, which gradually decreased overtime. Peak values for imaging were slightly higher for both basal flow and erythema in the Imiquimod group versus the UV-B group. In naïve, unchallenged skin, trace or mild C3d staining was observed in the

dermis, with no significant C3c deposition. No significant induction of C3c was seen at any time point after imiquimod or UV-B challenge.

Date and Version of this Report:

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