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ARTICLE

Intradermal substance P as a challenge agent in healthy individuals

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Abstract

Pharmacological challenge models are deployed to evaluate drug effects during clinical development. Intradermal injection of Substance P (SP) neuropeptide, a potential challenge agent for investigating local mediators, is associated with wheal and flare response mediated by the MRGPRX2 receptor. Although dose-dependent data on SP effects exist, full characterization and information on potential carryover effect after repeated challenge are lacking. This open-label, two-part, prospective enabling study of SP intradermal challenge in healthy participants aimed to understand and distinguish between wheal and flare responses following various SP doses. Part 1 included one challenge visit to determine optimum SP dose range for evaluation in part 2, which determined variability in 20 participants and used intradermal microdialysis (IDM) for SP-challenged skin sampling. At 5, 15, 50, and 150 pmol doses, respectively, posterior median area under the curve (AUC; AUC_{0-2h}) was 4090.4, 5881.2, 8846.8, and 9212.8 mm^2/min, for wheal response, and 12020.9, 38154.3, 65470.6, and 67404.4 mm^2/min for flare response (SP-challenge visit 2). When the challenge was repeated ~2 weeks later, no carryover effect was observed. IDM histamine levels were relatively low, resulting in low confidence in the data to define temporal characteristics for histamine release following SP challenge. No safety concerns were identified using SP. Wheal and flare responses following intradermal SP challenge were dose-dependent and different. The results indicate that this challenge model is fit-for-purpose in future first-in-human studies and further assessment of novel drugs

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targeting dermal inflammatory disease responses, such as chronic spontaneous urticaria, chronic inducible urticaria, and pseudo-allergic reactions.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Intradermal challenge with Substance P (SP) is known to cause wheal and flare response, which increase in size with increased doses of SP.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study investigated the feasibility of SP for proof-of-pharmacology trials in terms of test–retest variability and objective imaging characterization.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Wheal and flare responses following intradermal challenge with increasing doses of SP were found to differ, and limited carryover of effect was observed following repeated challenge. This supports the fit-for-purpose validation for application of the challenge model in future clinical assessment of novel pharmacological agents, that antagonize the induced wheal and flare response.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The use of SP in a challenge study could potentially aid in the investigation of locally acting mediators and this knowledge will aid in understanding the mechanistic pathways downstream of SP activation.

INTRODUCTION

Substance P (SP) is a neuropeptide that acts on mast cells in the skin, resulting in neurogenic inflammation[1] primarily through activation of mast cells via the Mas-related G-protein coupled receptor X2 (MRGPRX2) and neurokinin 1 receptor on endothelial cells.[2–5] Mast cell degranulation is the key pathophysiological event in diseases, including chronic spontaneous urticaria, chronic inducible urticaria, and pseudo-allergic reactions.[6–8] Although there are several other challenges available that are associated with MRGPRX2 signaling, such as somatostatin, proteases such as cathepsin S, and antimicrobial peptide insulin-like growth factor-binding protein 5 (AMP-IBP5), previous studies have demonstrated that SP plays a role in neurogenic inflammation and pain associated with wound healing.[8–11] A high-affinity MRGPRX2 antagonist has yet to be developed.[12]

SP is upstream in the inflammatory response signaling cascade and may be a useful challenge agent for the investigation of locally acting mediators in some settings.[3,13] Challenge models mimic pharmacologically induced conditions, providing a valuable tool to assess an inflammatory response in healthy human volunteers and analyze the potential efficacy of drugs in development before going to patient populations.[5,14–16] Increasing doses of SP via intradermal injection are associated with an increased wheal and flare response,[9–11] as well as intradermal release of several inflammatory mediators, such as histamine and tryptase. Histamine can be used as an active control versus SP, as histamine is an agent known to produce wheal and flare responses.[5] Although dermal challenges with SP are available and date back to the 1970s,[17] a detailed understanding of the effect of increasing SP dose on wheal and flare, characterization of doses over multiple timepoints, and histamine response, are lacking. There are no published results of pharmaceutical agents tested with this model as of yet.[10] Optimizing the intradermal challenge model will facilitate future clinical and pharmacological evaluation of antagonists to block or decrease the induced wheal and flare response. Novel compounds targeting MRGPRX2 would be one potential application. In addition, to our knowledge, the test–retest variability as well as potential for carryover of effect following repeated SP challenge has not previously been reported.

The aim of the current study was to elucidate the robustness of SP response by evaluating the effect of various SP doses on wheal and flare as end points related to MRGPRX2 receptor-mediated mast cell degranulation. MRGPRX2 is exclusively expressed on mast cells, is responsible for non-IgE-mediated mast cell activation, and has affinity for many molecules, including SP and various drugs.[8,18] As such, an SP challenge model may be used to evaluate drugs for inhibition of the MRGPRX2 pathway, which are designed to treat non-IgE-mediated diseases.

The study was conducted in two parts: the objective of part 1 was to select the correct SP doses suitable for further investigation in part 2. In this paper, the results of part 2 of the study will be explored in detail, with part 1 data outlined in the Supplement. In part 2, the effect of the SP dose on wheal and flare response was evaluated at two consecutive challenge visits. Intradermal microdialysis (IDM) was also performed in part 2 of the study. IDM involves the insertion of dialysis membranes into the dermis, which is then perfused at a low speed with the perfusate. Endogenous or exogenous molecules soluble in the extracellular fluid diffuse into the membrane and are then collected in small vials for analysis. By means of this technique, continuous sampling of interstitial fluid from SP-challenged skin is possible and allows for evaluation of an effect-time relationship.[17] Overall, this study aimed to develop an SP challenge model that is fit-for-purpose for future studies and to understand the mechanistic pathways downstream of SP activation.

METHODS

Study design

This was a single-center, two-part, prospective enabling study of SP intradermal challenge in healthy participants conducted between February and March 2021. An open-label design was chosen for operational considerations and because the main read-outs of wheal and flare were determined by the caliper method. The study was registered at ClinicalTrials.gov with the identifier NCT04676763; the study protocol was approved by the Ethics Committee of the Stichting Beoordeling Ethiek Biomedisch Onderzoek.

Parts 1 and 2 were conducted sequentially. In part 1 (Figure 1a), participants attended one challenge visit as outpatients, and in part 2 (Figure 1b), participants attended two challenge visits as outpatients and had one follow-up phone call. From screening to last follow-up visit, the duration of part 1 was up to 4 weeks, and the duration of part 2 was up to 7 weeks.

The intradermal SP challenge was administered sequentially from the lowest to the highest dose, as 5, 15, 50, and 150 pmol SP, respectively, at a volume of 50 µL. The SP doses were in line with previously published research[10]; we aimed to establish a dose–response relationship, therefore a 30-fold difference was selected for this study.

At each challenge visit, participants first received saline by intradermal injection, and histamine by skin prick, as negative and positive controls, respectively. The participant received SP if the wheal response met the acceptable saline and histamine response criteria, 20 min after each control challenge. The standardized interval of 20 min ensured that delayed responders could still participate, given that the effects of histamine are short-lived.[19,20] The acceptable responses were defined as saline wheal less than or equal to 1 mm or histamine wheal greater than or equal to 3 mm, primarily measured using the longest diameter of the wheal by calipers, which were readily available to clinical study sites for standard use. Following confirmation of acceptable saline and histamine control responses, participants received up to four intradermal injections of SP at different doses.

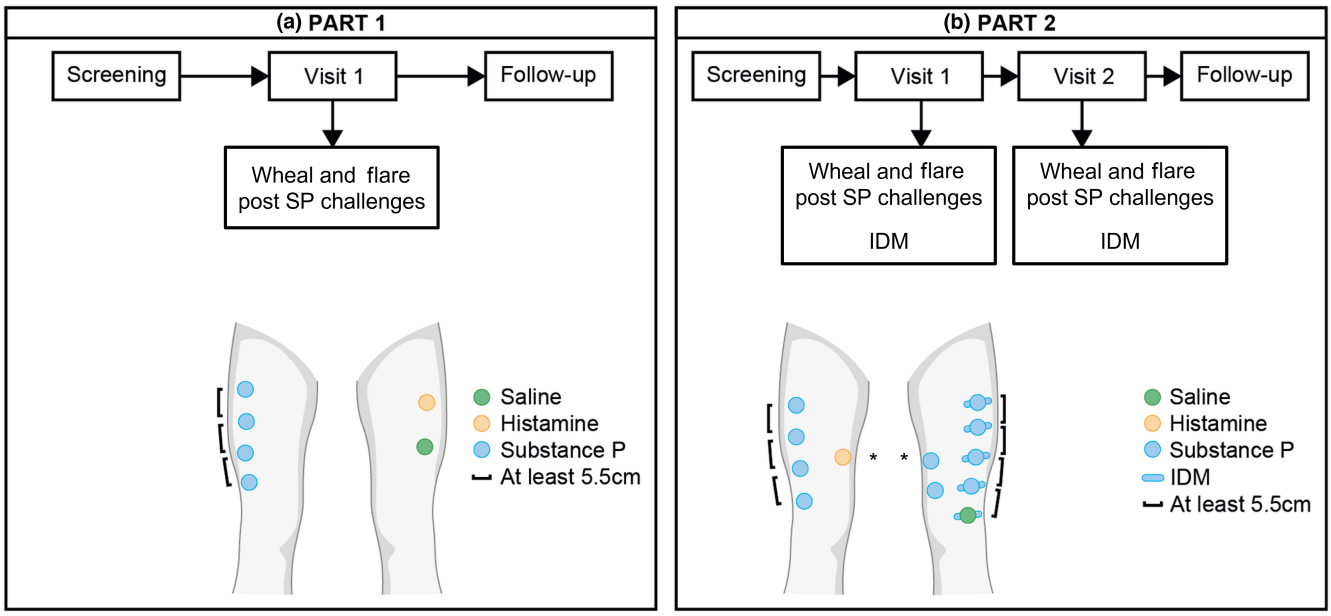

FIGURE 1 Study design. IDM, intradermal microdialysis; SP, substance P. *Asterisks refer to the positive histamine control that were performed at the back for the thigh. Figure was created using BioRender.com.

During part 2 of the study, participants also underwent IDM, which comprised an additional single injection of saline and four increasing doses of SP like those used in the flare and wheal challenges. IDM probes were inserted intradermally in the skin of the upper leg of participants (Figure 1b). In total, ~450 μL of dialysate was collected from each probe to measure histamine content in each participant. The IDM probes were inserted at least 2 h before the baseline sampling to reduce IDM procedure-induced wheal and/or flare. IDM samples were taken at the challenge site, before and after each challenge. The samples were taken up to 120 min post-SP challenge, however, an interim analysis on the first eight participants determined that samples taken past 30 min reached below the lower limit of quantification (LLOQ) values, and it was therefore decided to only analyze samples up to 40 min post-challenge for the remainder of the participants.

The histamine assay was validated on a fit-for-purpose principle: validation of the exploratory relative quantification of histamine concentration in IDM samples was performed as an extension of previous validation by RefLab ApS (Copenhagen, Denmark) of their in-house basophil histamine release assay. Extension of the validation for the context of use described here covered the IDM perfusate, IDM probes, and sample stability upon storage conditions required in this study. The LLOQ and upper limit of quantification of the assay were determined during the method validation extension. Data were included in the analysis only if they met predefined acceptance criteria based on variability between replicates and if they measured within the validated range of the assay.

Study population

Participants were recruited via advertisements on social media and a healthy volunteer database at the Centre for Human Drug Research, Leiden, The Netherlands. Eligible participants were men and non-pregnant women, 18–64 years of age, with Fitzpatrick skin type I–II, body weight greater than or equal to 50 kg, and a body mass index within the range of 19.7–29.4 kg/m^2. Participants were required to have a positive response to the histamine skin prick and a negative response to the saline injection at screening. Participants were excluded from the study if they had significant skin-related disorders, skin damage, or other disfiguration (tattoos, body piercings, and branding) on or near the site of application, which could interfere with assessments. Additional exclusion criteria included use of any form of H1 or H2 antihistamines, tricyclic antidepressants, beta-2 agonists, dopamine, or beta-blocking agents within 14 days of the first challenge, and individuals who were at risk or had previously experienced complications from a skin biopsy (including excess bleeding, infection, or scarring/keloid formation). Participants were also ineligible if they used topical medications and were unable to refrain from the use of topical medications from the first to the last challenge visit. Written informed consent was obtained from each participant prior to the performance of any study-specific procedures.

Study outcomes

The primary outcome measure was wheal response, which is the area in millimeters squared (mm^2) and was calculated using the formula for an area of ellipse with the longest and orthogonal diameters measured with calipers. The response was summarized in various secondary outcomes: area under the curve (AUC; mm^2/min) during the 2-h post-challenge period at each dose of SP,[21] maximum area of wheal, time taken to observe the maximum wheal area, and time to complete disappearance of wheal. The same secondary outcomes were investigated for flare response during the 2-h post-challenge period. Other secondary outcomes included incidence of adverse events (AEs) and incidence of laboratory or physical findings of clinical importance (including electrocardiogram assessment at screening, baseline, and post-challenge).

Statistical analysis

Descriptive summaries were calculated for the wheal and flares responses.

The statistical analysis of the SP-induced wheal/flare AUCs was conducted using the SAS software version 9.4 (SAS Institute, USA), using a Bayesian repeated measures model. Point estimates and associated variability were reported as posterior medians and 95% credible intervals.

To assess the dose-dependent response, we calculated the ratio of the SP-induced wheal/flare AUC between two consecutive doses of SP. A ratio exceeding one indicates an increase in the AUC between the two consecutive doses of SP. The confidence associated with this ratio was assessed by calculating the probability of that ratio being above one.

RESULTS

Baseline characteristics

Overall, 32 participants were enrolled, and 29 participants completed the study: nine participants in part 1 and 20 in part 2. Of the three participants who did not complete

the study, two were excluded due to a coronavirus disease 2019 (COVID-19) infection, and one voluntarily chose to withdraw. In total, 20 of 32 (62.5%) participants were women, and the median age (range) of participants was 22 (18–49) years. Additional details of the participant characteristics are presented in Table S1.

Wheal response

SP produced a dose-dependent wheal response in both parts of the study (Table 1), with the posterior median for SP-induced wheal AUC demonstrating a clear distinction between the different doses of SP (Figure 2). Please see Figure S1 and Table S2 for mean wheal AUC following skin challenges for part 1 of the study. In part 2 of the study, during visit one, the range in response was pronounced, with a posterior median that ranged between 4036.7 and 10011.0 mm^2/min, for 5 and 150 pmol of SP, respectively. A dose-dependent ratio of 1.5 was obtained between SP 15 and 5 pmol, signifying that the wheal AUC at SP 15 pmol was 1.5 times higher than the wheal AUC response at SP 5 pmol (Table 1). At visit two, the range in response was similar to the one observed at visit one: the posterior median ranged between 4090.4 and 9212.8 mm^2/min for SP 5 and 150 pmol, respectively (Table 1). At both visits, an SP-dependent wheal response was observed with most of the ratios greater than one. Please see Figure S2 for mean wheal AUC following skin challenges with IDM intervention.

The mean maximum wheal response increased with SP doses in both parts of the study (Figure 2). A summary of wheal responses following skin challenges is provided in Table S3. During part 2, 5 pmol of SP produced a mean maximum wheal response of 85.8 mm^2 and 92.2 mm^2 at visits one and two (at 20 min), respectively; this increased to 148.9 mm^2 and 153.0 mm^2, respectively. The mean time taken to achieve maximum wheal area was similar across all SP doses in both parts of the study and ranged from 20.4 to 31.7 min during visit one of part 2 (Table S3). The maximal effect (E_{max}) at 50 pmol of SP, with a median max area of 141.1 mm^2, was first observed at visit one during part 2 of the study (Table S3).

The mean time to maximum response during part 2 was similar across visits, ranging from 20.4 to 31.7 min for visit one and 20.9 to 28.3 min for visit two. When time to complete resolution of the wheal was evaluated, it was observed that the wheal area lasted longer with the highest dose of SP (90 min at 150 pmol). In part 2, at visit one, wheal area in four participants lasted longer than 2 h at SP doses of 50 and 150 pmol, whereas all wheal responses resolved within 2 h at the lower dosages; a similar trend was observed in part 1 (Table S2). In part 2, the SP-induced wheal responses were similar with and without IDM interventions (Figure 2 and Figure S2).

Flare response

SP produced a dose-dependent flare response, with the posterior median for SP-induced flare AUC demonstrating a distinction between the different doses of SP (Figure 3 and Table 1). Please see Figure S3 and Table S2 for mean flare AUC following skin challenges for part 1 of the study. In part 2 of the study, during visit one, the variability in response had a posterior median that ranged between 9070.7 and 67260.8 mm^2/min, for 5 and 150 pmol of SP, respectively. At visit two, the variability in response was more pronounced, with a posterior median ranging between 12020.9 and 67494.4 mm^2/min, for 5 and 150 pmol of SP, respectively (Table 1). A dose-dependent AUC ratio of 3.9 and 3.2 was obtained for SP 15 and SP 5 pmol at visit one and visit two, respectively (Table 1).

Please see Figure S4 for mean flare AUC following skin challenges with IDM intervention.

SP challenges were associated with a rapid onset of flare response within 5 min after SP administration (Figure 3). For both parts of the study, a dose-dependent flare response was observed between the 5-min and 20-min timepoints with a peak time to maximum response observed between 5 and 10 min (Figure 3 and Table 1). Intra-participant variability in flare response was observed between visits one and two of part 2 of the study, resulting in a difference in mean flare areas being observed between the two visits (Table 1). In both parts of the study, there was a saturation effect at 50 pmol of SP (E_{max} effect). The IDM interventions did not change SP-induced flare responses, and all flare responses were resolved at the same timepoint of 90 min (Figure 3).

IDM histamine analysis

Mean histamine concentrations measured in part 2 IDM samples were detectable 0–30 min following an SP challenge of at least 15 pmol, with a peak response at 10 min (Figure S5). Histamine concentrations were increased in a dose-dependent manner to SP challenges of 15, 50, and 150 pmol, although there was variability in response. Histamine levels were not detectable (values below LLOQ, 10 ng/mL) 0–30 min following saline and 5 pmol of SP. Histamine concentrations were also less than the LLOQ at all timepoints after 30 min for all challenges tested. For the first eight participants included in the interim analysis, there was no measurable histamine following saline and 5 pmol of SP challenge. At 50 pmol of SP, there was a

TABLE 1 Summary of wheal and flare AUC following skin challenge with ascending Substance P doses.

Challenge visit	SP challenge	Posterior median (mm^2/min)	95% CrI (lower, upper)	Probability (ratio >1)[a]
Wheal AUC_{0-2h} (part 2)				
Visit 1	Dose, number of participants			
	SP 5 pmol, $n=20$	4036.7	(3209.7, 5061.7)	
	SP 15 pmol, $n=20$	5961.9	(4746.6, 7496.7)	
	SP 50 pmol, $n=20$	7914.1	(6297.6, 9946.0)	
	SP 150 pmol, $n=20$	10011.0	(7970.2, 12555.9)	
	AUC ratio[b]			
	SP 15 pmol/SP 5 pmol	1.5	(1.1, 2.0)	1.0
	SP 50 pmol/SP 15 pmol	1.3	(1.0, 1.8)	1.0
	SP 150 pmol/SP 50 pmol	1.3	(0.9, 1.8)	0.9
Visit 2	Dose, number of participants			
	SP 5 pmol, $n=20$	4090.4	(3357.9, 4979.9)	
	SP 15 pmol, $n=20$	5881.2	(4841.5, 7159.4)	
	SP 50 pmol, $n=20$	8846.8	(7276.5, 10766.0)	
	SP 150 pmol, $n=20$	9212.8	(7563.1, 11216.9)	
	AUC ratio			
	SP 15 pmol/SP 5 pmol	1.4	(1.1, 1.9)	1.0
	SP 50 pmol/SP 15 pmol	1.5	(1.1, 2.0)	1.0
	SP 150 pmol/SP 50 pmol	1.0	(0.8, 1.4)	0.6
Flare AUC_{0-2h} (part 2)				
Visit 1	Dose, number of participants			
	SP 5 pmol, $n=20$	9070.7	(4984.8, 16493.6)	
	SP 15 pmol, $n=20$	35302.2	(19496.2, 64444.1)	
	SP 50 pmol, $n=20$	59651.7	(32994.9, 108116.6)	
	SP 150 pmol, $n=20$	67260.8	(36810.8, 121853.7)	
	AUC ratio[b]			
	SP 15 pmol/SP 5 pmol	3.9	(1.7, 9.1)	1.0
	SP 50 pmol/SP 15 pmol	1.7	(0.7, 4.0)	0.9
	SP 150 pmol/SP 50 pmol	1.1	(0.5, 2.6)	0.6
Visit 2	Dose, number of participants			
	SP 5 pmol, $n=20$	12020.9	(6368.7, 22514.5)	
	SP 15 pmol, $n=20$	38152.3	(20522.4, 71413.2)	
	SP 50 pmol, $n=20$	65470.6	(34927.1, 123048.7)	
	SP 150 pmol, $n=20$	67404.4	(36201.0, 126680.1)	
	AUC ratio[b]			
	SP 15 pmol/SP 5 pmol	3.2	(1.3, 7.7)	1.0
	SP 50 pmol/SP 15 pmol	1.7	(0.7, 4.2)	0.9
	SP 150 pmol/SP 50 pmol	1.0	(0.4, 2.5)	0.5

Abbreviations: AUC, area under the curve; CrI, credible interval; SD, standard deviation; SP, Substance P.

[a]Posterior probability that the AUC ratio is >1.

[b]The AUC ratio is the ratio of the AUC between two consecutive doses of SP. A ratio exceeding one indicates an increase in the AUC between the two consecutive doses of SP.

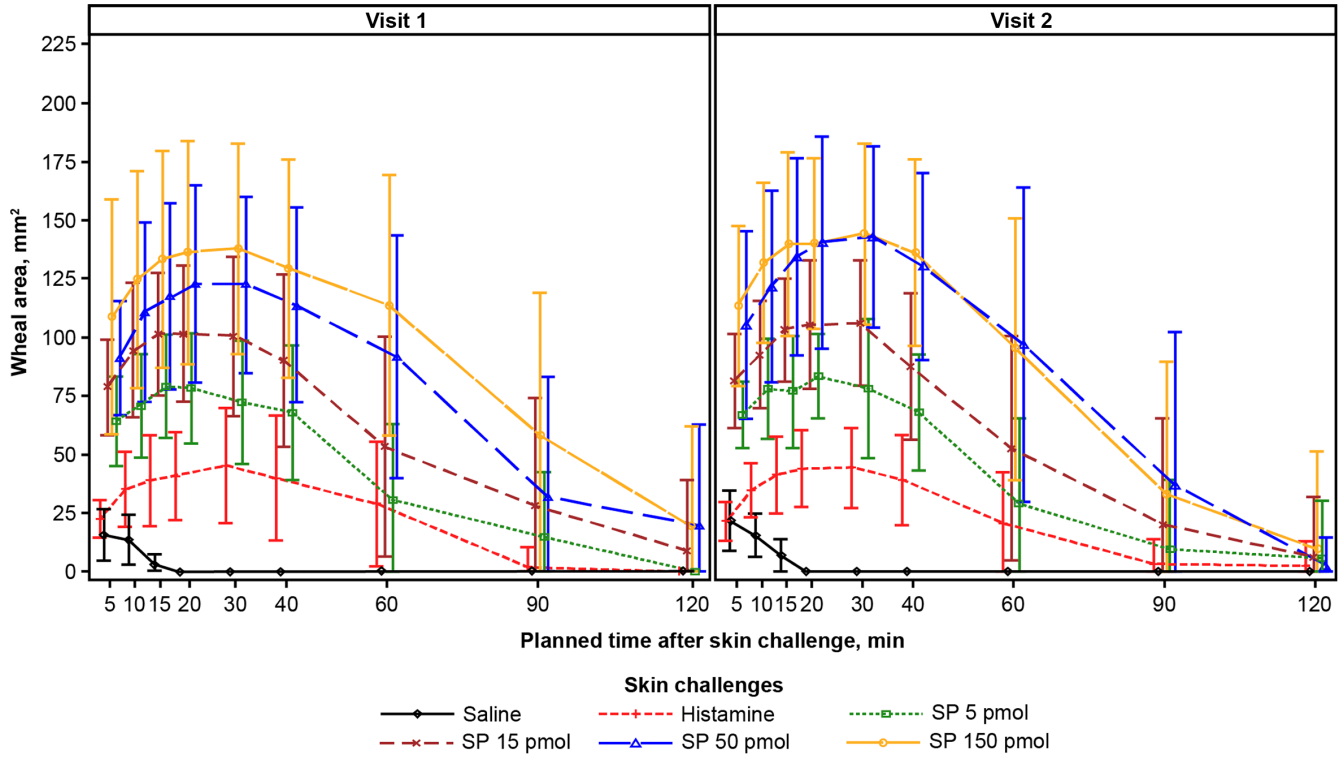

FIGURE 2 Mean wheal AUC following skin challenges (Part 2, non-IDM). AUC, area under the curve; IDM, intradermal microdialysis; min, minutes; SP, substance P.

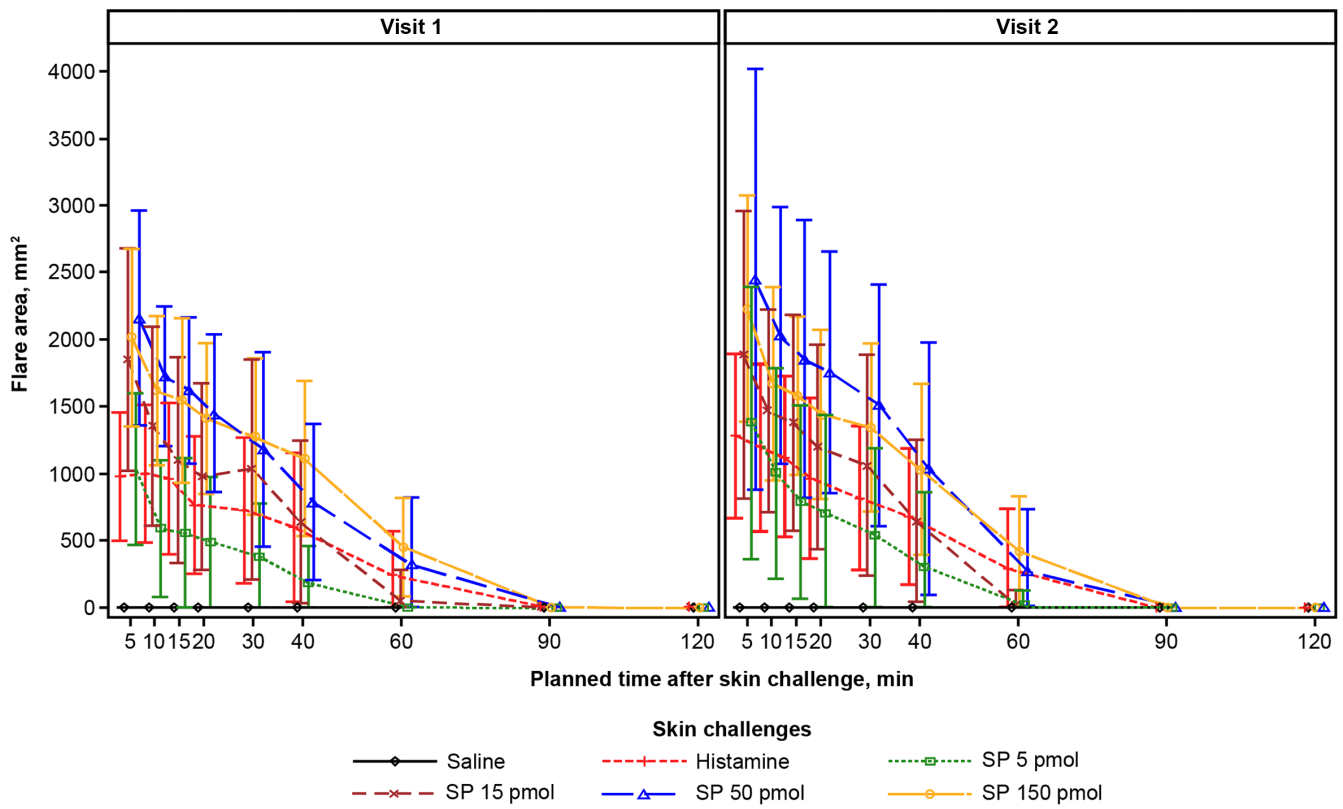

FIGURE 3 Mean flare AUC following skin challenges (Part 2, non-IDM). AUC, area under the curve; IDM, intradermal microdialysis; min, minutes; SP, substance P.

relatively small histamine response at 10 and 20 min for a subset of participants at visit two only. A higher proportion of subjects had detectable histamine levels with 150 pmol of SP challenge in comparison to 50 pmol (Figure S5).

Safety

AEs of mild intensity were experienced by one participant (11%) in part 1 (pruritus) and two participants (10%) in part 2 (headache). The pruritus AE in part 1 was considered SP-related, whereas the AEs in part 2 were considered unrelated to SP administration. No moderate or serious AEs were experienced by any of the participants, and no major safety concerns were observed within the clinical laboratory parameters. In addition, no clinically significant electrocardiogram abnormalities were observed.

DISCUSSION

The time profile of the challenge for wheal and flare responses provides greater insight and characterization of the effect of multiple doses of SP over multiple timepoints, in addition to determining the potential carryover effect following repeated challenge. In this study, lower doses of SP produced dose-dependent wheal and flare responses over the 2-h post-challenge period, as measured by the AUC in both parts of the study with low test–retest variability. The safety and tolerability of the challenge agent was acceptable, with only a small number of mild AEs occurring. These findings are consistent with a previous study, which looked at the effect of SP on wheal and flare on human skin.[2] The large maximum wheal response of 153 mm^2 increased with increasing doses of SP, and wheal response lasted only 90 min with the highest SP dose.

Wheal and flare reactions can be induced by SP through intradermal injection or skin prick testing. Antihistamines can suppress these reactions, suggesting SP activates skin mast cells to release histamine.[22] In a study by Fujisawa et al. using skin-derived cultured mast cells, the MRGPRX2 receptor was found to be responsible for histamine release induced by SP instead of the traditional SP receptor NK-1R.[23]

The present study sought to determine the dose–response and the robustness of the SP challenge model, by assessing the effect of ascending dose of intradermal SP on wheal and flare responses in healthy participants. This study also aimed to establish and validate an SP intradermal challenge model to allow the future clinical evaluation of the pharmacological ability of MRGPRX2 receptor antagonists to block or decrease the induced wheal and flare response; in a single- or multiple-ascending-dose trial, the challenge would be used to demonstrate functional target engagement in humans.[24]

The study was designed to determine the potential carryover of effect and the outcome. Wheal and flare responses following intradermal challenge with increasing doses of SP were found to differ, and limited carryover of effect was observed following repeated challenge: baseline visits were comparable between visit one and visit two and no differences were observed before dosing. The SP challenge model does not represent a disease model, but a pharmacological model that can establish engagement and proof-of-pharmacology in a first-in-human setting. This supports the potential use of the challenge model in future clinical assessment of novel pharmacological agents that antagonize the induced wheal and flare response, for example, during future clinical assessments of novel agents in healthy volunteers before evaluation in patients with diseases such as chronic spontaneous urticaria, rosacea, and atopic dermatitis, caused by activation of MRGPRX2 receptor.[25] Other examples of pharmacological intradermal challenge models include the histamine challenge model, the imiquimod model and the lipopolysaccharide model of inflammation.[15,26,27] In the current study, the SP challenge model was characterized and the results can be assessed as fit-for-purpose validation of this method.[28]

Our study also evaluated flare responses and demonstrated that SP challenges produced a dose-dependent flare response; flare response lasted longest (between 5 and 10 min) with the highest dose of SP. The results are supported by previous findings, in which SP was observed to induce a dose-dependent wheal and flare for the lower doses.[2,10,29,30] Notably, our findings show an E_{max} at SP 50 pmol in the first challenge visit. Further, we found that there was variability in both the wheal and the flare AUCs observed with different doses. A dose-dependent flare response was observed between the 5- and 20-min timepoints, which coincided with findings from the published literature: response during the first 5 min was reported to be the most reproducible period to assess the inflammatory response.[31]

For the majority of participants, responses disappeared within 2 h following SP administration. Only a few lasted longer, and individuals with longer-lasting responses were excluded from the analysis. However, it must be noted that this analysis was conducted with a small number of participants, and it is unclear if similar trends would manifest in larger studies. Nonetheless, the relatively small sample size in the present study was similar to that utilized in a prior challenge study,[31] and suggests that the skin challenge is suitable for small cohorts in the early phases of clinical development. One could argue that this study may have been limited by its open-label design; however, controlling for the risk of bias was not required for this type of design and appropriate within-subject controls were used.

Our study showed that IDM interventions did not change SP-induced wheal and flare responses, and this further supports the use of the SP model. The concentration of histamine in the clinical samples, released in response to SP challenge, was lower than expected based on previous findings.[32] These relatively low concentrations of histamine may have been due to a rapid local clearance of histamine in the skin after the mediator was released from the skin mast cells. This hypothesis was consistent with the often rapid decline in histamine concentrations observed when two consecutive samples from the same probe were positive, that is, above the LLOQ. This rapid clearance of histamine following SP challenge in skin has previously been reported,[32] similarly with a peak response measured up to 10 min.

In conclusion, we demonstrate that a pharmacological model with SP in healthy participants should be considered for proof-of-pharmacology analyses in first-in-human studies. The use of SP in a challenge study could enable researchers to investigate the release of locally acting mediators, in addition to immune cell markers.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. W.tV., C.A., I.A., S.W., R.R., R.K., K.F., L.F., C.M., S.M., K.B., M.vD., and R.B. designed the research. W.tV., S.W., N.K., M.B., M.vD., and R.R. performed the research. W.tV., C.A., S.W., R.R., R.K., S.H., A.N., L.F., S.M., K.B., M.vD., and R.B. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

W.tV., I.A., S.W., N.K., M.B., M.vD., and R.R. are employees of the Centre for Human Drug Research which received funding from GSK to conduct this study. C.A., R.K., K.F., A.N., L.F., C.M., S.M., and R.B. are employees of and shareholders in GSK. K.B. is an employee of RefLab ApS which received funding from GSK to conduct this study. S.H. was an employee of and shareholder in GSK at the time of study.

DATA AVAILABILITY STATEMENT

Information on GSK's data sharing commitments and requesting access to anonymized individual participant data and associated data can be found at www.clinicalstudydatarequest.com.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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