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Skin Response to Histamine

Reproducibility Study of the Dry Skin Prick Test Method and of the Evaluation of Microvascular Changes with Laser Doppler Flowmetry

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The author presents original data obtained when using the dry skin prick test method to introduce histamine into the skin and by non-invasive evaluation of the skin blood flow changes at various sites/times during the development of the weal and flare reaction. The test method (1-second prick test duration and evaluation with laser Doppler flowmetry) generated reproducible responses when repeated in the same group of subjects (n = 10). At predefined fixed skin locations within the histamine-induced flare reaction area increased volumes of skin blood flow were recorded. When similar locations were explored after the control prick test, there were only minimal changes in skin blood flow. Pooled data recorded at four different sites located 1 cm from prick sites showed minimal variation and skin perfusion volumes were greater than basal values. The development of the histamine-dependent weal in its early phase (9th min) was associated with slightly smaller numbers of skin blood perfusion units, compared with recordings made during the 15th min. The control prick tests showed slightly higher levels at 9 min than at 15 min. This inverse relationship might be useful to quantify the histamine-specific changes in skin blood flow. These data also clearly illustrate that it is mandatory to state the precise place of measurement and time after challenge when reporting on instrumental evaluation of the skin response to histamine. ***Key words: Pharmacology; Non-invasive methods; Evaluation methodology.***

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In recent years, laser Doppler flowmetry (LDF) has been used to evaluate skin reactions following intradermal injection of histamine. The measurement by LDF of cutaneous blood flow at 1 cm from the site of histamine injection appeared to generate the most reliable data (1, 2). The severity of microinjury and the speed and volume of the injected material are potential factors of variability in measurements made at injection sites (3–5). In order to standardize agonist administration as much as possible, we used the dry skin prick test (Phazet™, Pharmacia AB, Sweden), a ready-to-use disposable material. When this material was used in previous experiments, a single site was probed at a distance from the prick, in order to measure skin blood perfusion in the flare reaction. The degree of reproducibility was acceptable (5, 6).

A point that has not been previously considered in great detail is the reproducibility of multiple measurements made at 1 cm from the histamine administration site during specific periods of time during the development of the weal and flare reaction. We report here the results of a comparative study designed to evaluate the reproducibility of skin blood flow measured at a distance from (1 cm proximal, distal, external and internal from the prick test) and at the site of skin prick tests, using the histamine and control dry skin prick tests.

MATERIAL AND METHODS

Subjects

Ten adult subjects (average age 21,3 years; 6M and 4F) volunteered for this study which was approved by the medical ethics committee on human investigation (IMC, Tournai, Belgium). These subjects had no personal history of atopic skin or respiratory disease. The site used in this investigation was located 5 cm from the elbow, on the volar aspect of the forearm.

Skin prick tests

Histamine was administered using the dry skin prick test method, which means that no solution is applied to the skin surface and that nothing other than histamine is introduced into the dermis (0.79 ± 0.05 mm; average of ten penetrating edges). The disposable prick needle (generously supplied by Pharmacia AB, Sweden) was firmly pressed into the skin at 90° and held in that position for 1 s. Prick tests with unloaded needles were used as a negative control.

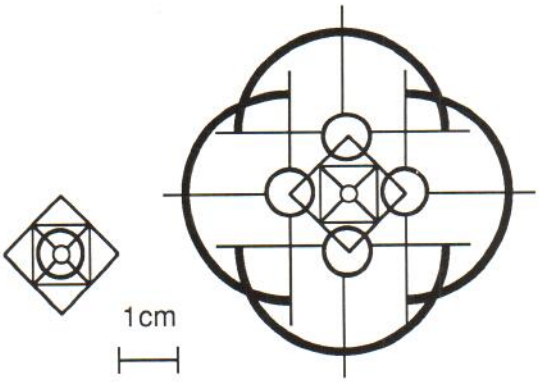

Fig. 1. Schematic view of laser Doppler probeholders for measurement at fixed skin locations. Five commercially available standard probeholders have been used and were reassembled in order to match this model. The fit of the central holes where the probe is inserted was checked at regular intervals during the trial. There was no significant drift in the relative position of each measurement point relative to the central probeholder.

Skin blood perfusion measurements

The measurements were performed by laser Doppler flowmetry (LDF; Periflux PF3, Perimed, Sweden). This instrument displays continuously the intensity of skin perfusion units (PU) on a digital screen. The LDF probe was inserted into a commercially available probe holder. For the specific purpose of this experiment, the central part of five such probe holders was cut out and reassembled in such way as to provide the constant 1 cm distance/orientation between the central and peripheral measurement points (Fig. 1). The central measurements site was carefully overlaid onto the prick test site under visual control through the hole of the central probe holder and once the whole assembly was correctly oriented it was fixed to the skin surface with double-sided adhesive tape (Tesafix, Beiersdorf AG, Germany).

Experimental design

Experiment 1: Comparison of histamine and control prick test responses. During this first experiment (exp 1), after measuring basal blood flow on the right forearm (1 min), a dry skin histamine prick test was made 5 cm from the elbow fold. Five minutes later, probing of the skin vascular response was started. For each series of measurements, after a stabilization period of 10 s, readings of skin blood perfusion were made. The displayed perfusion units were recorded every 5th s for a period of 50 s. The probe was then moved to the next point and readings were started again after 10 s. A first dataset was recorded as described on preprinted data recording sheets in the following sequence and at 1 cm from the administration site: proximal (5th min), external (6th min), distal (7th min) and internal (8th min). Finally, the last measurement of the first set of recordings started during the 9th min and was made exactly at the site of histamine administration. A second set of measurements were recorded between the 11th and 14th and during the 15th min after prick testing, respectively at a distance from and at the skin prick test site. As a control, we performed, on a symmetrical site on the left forearm, a skin prick test with an unloaded needle. The measurements were made in exactly the same way as for the histamine-loaded needles.

Experiment 2: Reproducibility study of the dry skin prick test induced histamine response. In order to evaluate the reproducibility of the measurement method, the histamine dry skin prick tests were repeated in the same subjects (exp 2) within 10 d (on average) of concluding the first experiment. This time, two prick tests were studied on symmetrical sites on the proximal area of flexural aspect of the left and right forearm (slightly external to the site used in exp 1). The test method was as described above, with the exception that only the first data set was recorded (from 5 to 9 min). Indeed, in earlier experiments, skin perfusion data at a distance from the histamine dry skin prick tests appeared to remain stable between 2 and 20 min (2) and the same pattern was recorded again during exp 1 (this study) using an updated laser Doppler flowmeter (PF3 instead of PF1) between 5–8 and 11–14 min.

Statistical analysis

Statistics shown in Fig. 2 are the average and the standard deviation of log-transformed data. Skin perfusion units (PU), were compared with paired *t*-test. The differences were considered significant when $p < 0.05$ (two-tailed, unless specified otherwise).

RESULTS

Basal values and changes observed after histamine and control prick test

There was no difference between basal values measured on left and right sides before testing. For the histamine dry skin prick test, at all sites and time points, there was no significant difference between the two sets of data recorded at a distance from the test site, i.e. those recorded during the 5–8 or 11–14 min periods (Fig. 2). The variations in PU around the average (1 min continuous recordings; untransformed data) were within biologically acceptable limits (20 sets of data from expts 1 and 2 after histamine: coefficient of variation (CV%) range: 1 cm: 2.8–4.8% and prick site: 5.69–7.27%; 10 sets of data from exp 1 after control: CV% range: 1 cm: 9.7–16.1% and prick site: 4.8–8%). When transformed data of blood perfusion units were pooled (10 individuals), we observed coefficients of variation below 13% or 25% after histamine challenge and control prick test, respectively. Around the control prick test site there was an increase in PU during 5–8 min as compared with 11–14 min ($p < 0.02$). This was attributable particularly to higher values during the 5th and 6th min ($p < 0.03$). Highly significant differ-

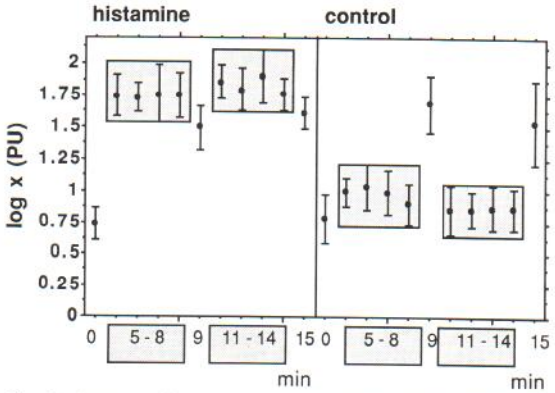

Fig. 2. Average (± 1 SD) of skin blood flow (perfusion units (log × (PU)) and changes observed after histamine (*left*) and control (*right*) prick test as a function of time. Basal blood flow values measured before testing (bars at time 0), were below 10 PU (1 on the log scale).

Histamine dry skin prick test responses: At a distance from the prick test, in the histamine-induced flares, increased blood flow levels were recorded ($p < 0.0001$). These sites were explored during the 5–8 and 11–14 min periods (outlined areas). Skin PU were recorded at 1 cm distance from the prick test site (proximal, external, distal and internal to the prick test site during the 5th and 11th, 6th and 12th, 7th and 13th, 8th and 14th min, respectively). During the 9th and 15th min, recordings were made at sites covered by the central part of the probeholder (Fig. 1) which happens to coincide with the prick test site. There was no significant difference between the sites explored during the 5–8 min or 11–14 min periods after histamine prick test (outlined areas, left). At prick test sites (9th and 15th min) values were increased as compared with basal levels ($p < 0.0001$). The histamine-induced weal was associated with lower skin blood flow values at 9 min as compared with those recorded at 15 min ($p < 0.03$).

Control prick test responses: After control prick tests (shaded areas, right), early recording times showed positive changes over basal recordings (5 and 6 min data; $p < 0.02$). All data around control prick tests were significantly lower than the data in the histamine-induced flares ($p < 0.0001$). At control prick test sites, values were increased as compared with basal data ($p < 0.0001$). However, as compared with the histamine-induced response, the reverse sequence was observed at control prick test sites, i.e. higher values at 9 min than at 15 min ($p < 0.05$).

ences were obtained when the surroundings of the histamine prick test data were compared with control data (see below).

Comparison of measurements at a distance from prick test site

The measurements of skin blood flow at 1 cm from the histamine dry skin prick test site differed significantly from basal levels and from those recorded at comparable site/time points after the control prick test ($p < 0.0001$), indicating an increase at all times and sites in the histamine-induced flare. A moderate but significant increase in blood flow values was recorded during the early recording times (5th and 6th min) around the control prick test site ($p < 0.02$). On average, untransformed values recorded at sites of histamine-induced flare were 6–9 times higher at 1 cm from the injection site than at homologous control prick test sites.

Comparison of measurements performed at prick test sites

Blood perfusion in skin prick test sites was increased over basal values ($p < 0.0001$). At the site of the histamine prick test (Fig. 2), there was a gradual increase in skin blood perfusion levels between 9 and 15 min ($p < 0.03$), while skin blood flow at control prick test sites decreased within the same time limits ($p < 0.05$).

Reproducibility study of histamine-induced skin responses

The coefficient of variation was similar from one experiment to another. There was no significant difference between skin blood flow measurements at comparable locations after histamine administration at various skin test sites (exp 1 right forearm; exp 2 left and right forearm).

DISCUSSION

Increases in skin blood flow are known to occur in the weal and in the erythematous flare associated with histamine administration in the skin (1–6). The repeated recordings on the digitized screen of the PF3-laser Doppler flowmeter were evidently more acccurate than the rough estimation of skin perfusion units (PU) on the PF1-voltmeter (2, 5, 6). It appears from this study that there is minimal variation of the PU within a given group of individuals when four different measurement points of the erythematous flare are explored (< 13% for the histamine dry skin prick test for the points located at exactly 1 cm from the prick test site). This indicates that precise locations should be reported in trials using these methods for evaluating agonist–antagonist interactions; for the 1 cm selected distance, we confirm that recordings are stable over a 15-min period of time (2) and that reliable measurements can be recorded at 5 min after prick testing. On average, there was no significant difference between

measurement performed at 1 cm from the histamine dry skin prick test site between the 5th and the 15th minute after injecting the agonist. Slightly elevated skin PU recordings were noted at early times (5th and 6th min) around the control prick test site (max. 2-fold increases over basal values as compared with 10-fold increases at comparable sites and times after histamine).

When measured at the prick test site, values of skin perfusion observed were lower 9 min after histamine administration than after 15 min, whereas levels recorded 9 min after the control prick test were higher than those recorded at 15 min. This might indicate that the blood flow increases normally observed at prick test sites were masked by the weal reaction; blood flow at later times, e.g. 15 min after prick tests, continued to increase as the weal spread out and faded away. The explanation put forward for this phenomenon is that extravasated fluid restricts blood supply at the site of histamine administration. This view was expressed earlier when we reported that, after ingestion of anti-H1 antihistamines, at dry skin prick test sites and at sites injected with histamine solution, there was a tendency to greater skin blood flow than following placebo (5, 6); in this case, the negative effect of edema on the vasodilation was suppressed by the H1 antagonists. The present data suggest that early recordings following histamine administration might be even more appropriate for measuring specifically the histamine-induced response.

REFERENCES

1. Serup J, Staberg B. Quantification of weal reactions with laser Doppler flowmetry. Allergy 1985; 40: 233–237.
2. Van Neste D, Coussement C, Rihoux JP. Validation d'une nouvelle méthode d'évaluation de la réponse cutanée à l'histamine et intérêt pour l'étude comparative de substances antihistaminiques. Nouv Dermatol 1989; 8: 51–57.
3. Shall L, Marks R. Non-invasive instrumental techniques to detect terfenadine and temelastine induced suppression of histamine induced weals in man. Br J Clin Pharmacol 1987; 24: 409–413.
4. Maurice PDL, Barr RM, Koro O, Greaves MW. The effect of prostaglandin D2 on the response of human skin to histamine. J Invest Dermatol 1987; 89: 245–248.
5. Van Neste D, Rihoux JP. Inhibition of the cutaneous response to histamine by H1-blocking agents. A quantitative evaluation of microvascular changes in the skin after histamine challenge and a comparison of the effects of a single intake of cetirizine and terfenadine. Skin Pharmacol 1988; 1: 192–199.
6. Rihoux JP, Van Neste D. Quantitative time course study of the skin response to histamine and the effect of H1 blockers. A three weeks crossover double-blind comparative trial of cetirizine and terfenadine. Dermatologica 1989; 179: 129–134.