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 (Phazer™, 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 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.
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 PFI) 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 exps 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-
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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 flare, increased blood flow levels were recorded ($p < 0.0001$). The 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 flare 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 ($p < 0.05$).

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 ($p < 0.05$).

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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 accurate 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

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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