

Quantification of Weal Reactions with Laser Doppler Flowmetry

Comparative Blood Flow Measurements of the Oedematous Centre and the Perilesional Flare of Skin-Prick Histamine Weals

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Skin-prick experiments with different concentrations of histamine (0.1 mg/ml, 1.0 mg/ml, 10.0 mg/ml) and isotonic NaCl solution for control were performed on the forearms of 10 healthy volunteers. Regular recordings of the cutaneous blood flow by laser Doppler flowmetry were performed in the centre of the weals and 5, 10, 15 and 20 mm from the centre representing the zone of perilesional flare. In the weal centre the blood flow was increased but neither different concentrations of histamine nor the control reaction could be differentiated. Recordings performed 5 and 10 mm from the centre showed, however, significant differences between the different solutions of histamine tested as well as the control reaction. The better differentiation was obtained 5 and 10 min after skin-prick. In conclusion, laser Doppler flowmetry with recordings of the perilesional flare is found useful for quantification of weal reactions.

Key words: allergy; blood flow; flare; hive; laser Doppler; oedema.

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Dilatation of the cutaneous vasculature and change in colour is essential in the histamine triple-response. The vasodilatation of the centre of weals, associated with formation of oedema, is mediated by histamine, while the perilesional flare is considered an axonal reflex. Recently, we studied diameters, thickness, skin surface area, cross-sectional area and volume of skin-prick histamine weals with high-frequency ultrasound (4). These parameters of cutaneous oedema allowed differentiation between weal reactions induced by different concentrations of histamine. In the present study the blood flow in the centre of weals as well as the perilesional flare is assessed by laser Doppler flowmetry in order to find whether this objective variable allows a more accurate evaluation of weal reactions.

MATERIAL AND METHODS

Ten healthy volunteers (7 women, 3 men) participated in the study. Their mean age was 40.9 years (33-59 years).

Skin-prick tests were performed on the flexor aspect of both forearms. Three tests with different concentrations of histamine chloride (0.1 mg/ml, 1.0 mg/ml, 10.0 mg/ml) and a control with sodium chloride (9 mg/ml) were performed in each person. The disposable "allergy prickler" of Dome/Hollister-Stier® with a point length of 1.0 mm was used, jabbed by moderate force through the drop of test solution at an angle of 90° to the skin and lifted, and the drop wiped off (3).

The blood flow of the weal reactions was recorded 5, 10, 15, 20 and 30 min after skin-

prick with the Periflux® laser Doppler flowmeter (8). Measurements were performed at the centre of the weal and 5, 10, 15, and 20 mm from the centre.

The light from the laser is led by an optical fibre to the skin surface and is backscattered with Doppler-shifted frequencies from moving blood cells and with unshifted laser frequencies from stationary tissues. The new system collects parts of the reflected light by using two optical fibres instead of one, and by this method the signal-noise ratio is increased. The signal is proportional to flow velocity. The wavelength of this helium-neon laser is 632.8 nm. The depth

of penetration of the light in skin is about 1 mm, and the illuminated skin surface area is about 2 mm². The instrument was adjusted to a bandwidth at 4 kHz and a gain at 10. The integrator was set at a high value to smooth out fluctuations due to the cardiac cycle.

The patients were examined in the horizontal position. The laser-Doppler probe was gently held to the skin by the examiner. In this way compression of the skin with its vasculature was minimized. The blood flow was measured for a period of 10–20 s, and the average blood flow was recorded. The tissue background (noise from the laser, or an output due to redistribu-

Table 1

Number of weals and perilesional flare reactions recorded during each experiment (10 persons tested)

	5 min		10 min		15 min		20 min		30 min	
	Weal	Flare	Weal	Flare	Weal	Flare	Weal	Flare	Weal	Flare
Sodium chloride control	2	1	3	1	5	1	5	0	3	0
Histamine 0.1 mg/ml	6	2	6	4	7	4	7	3	5	3
Histamine 1.0 mg/ml	9	9	10	9	10	9	10	9	10	9
Histamine 10.0 mg/ml	10	10	10	10	10	10	10	10	10	10

Table 2

Mean diameter and SD (bracket) in mm of weals and perilesional flare reactions as recorded during the experiments

Histamine	5 min		10 min		15 min		20 min		30 min	
	Weal	Flare	Weal	Flare	Weal	Flare	Weal	Flare	Weal	Flare
0.1 mg/ml	2.2 (2.4)	2.2 (5.4)	1.3 (1.3)	2.5 (3.3)	2.4 (1.8)	2.2 (2.9)	2.0 (1.9)	1.2 (2.0)	1.0 (1.4)	1.5 (2.6)
1.0 mg/ml	2.4 (1.5)	19.2 (9.8)	3.2 (1.5)	20.7 (10.8)	3.3 (1.4)	19.0 (10.7)	3.5 (2.2)	18.2 (12.4)	2.8 (1.9)	16.5 (10.4)
10.0 mg/ml	3.9 (1.5)	29.8 (4.8)	5.7 (1.7)	32.6 (4.3)	5.9 (1.7)	33.8 (6.5)	5.9 (1.9)	31.8 (6.6)	5.9 (1.6)	32.8 (7.8)

Table 3

Comparison of measurements of weal diameter and diameter of the perilesional flare reaction during the experiments – levels of significance

	5 min		10 min		15 min		20 min		30 min	
	Weal	Flare	Weal	Flare	Weal	Flare	Weal	Flare	Weal	Flare
Sodium chloride control/ histamine 0.1 mg/ml	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Histamine 0.1 mg/ml/ 1.0 mg/ml	NS	0.01	0.01	0.01	NS	0.01	NS	0.01	NS	0.01
Histamine 1.0 mg/ml/ 10.0 mg/ml	0.01	0.05	0.01	0.05	0.01	0.05	0.05	0.05	0.01	0.01

tion of RBCs in the capillaries) was measured on the skin of one arm 2 min after the blood flow had been stopped by a tourniquet applied proximally. This background value was subtracted from all measurements. Results were expressed in arbitrary units = volts. Room temperature was kept constant (20 to 22°C) during the entire trial.

The larger diameter of the weal as well as the perilesional flare were measured by a precision steel ruler with an accuracy of 1 mm.

Statistical analyses were performed by the Wilcoxon signed test for paired observations and by the Spearman rank correlation. Probabilities < 0.05 were considered significant.

RESULTS

The number of visible weals and perilesional flare reactions in each experiment, and the mean diameter and SD of weals and perilesional reactions appear in Tables 1, 2. Conclusions of statistical analysis of diameter measurements are presented in Table 3. None of the diameter recordings were at any time able to differentiate the sodium chloride control from histamine 0.1 mg/ml reactions. Weaker concentrations (0.1 and 1.0 mg/ml) of histamine could be differentiated by the flare diameter. Stronger concentrations (1.0 and 10.0 mg/ml) could be differentiated by the weal diameter, and after 10

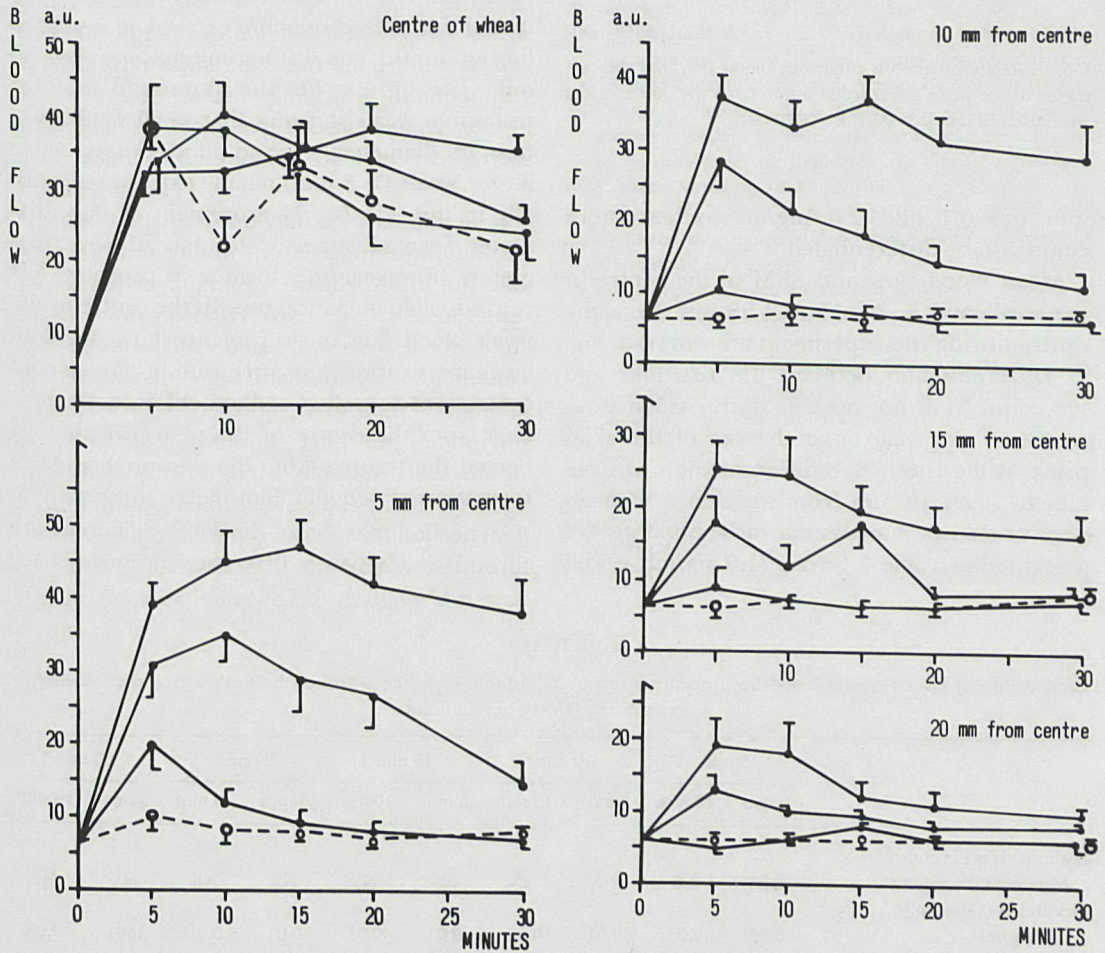


Fig. 1. Laser Doppler flow (mean and SEM) of histamine weals and sodium chloride control in the centre of the weal or at the site of the prick, and 5, 10, 15, and 20 mm from the centre as recorded after 5, 10, 15, 20 and 30 min. Symbols: $\circ$ sodium chloride control, $\bullet$ 0.1 mg/ml histamine chloride, $\blacksquare$ 1.0 mg/ml histamine chloride, $\blacktriangle$ 10.0 mg/ml histamine chloride.

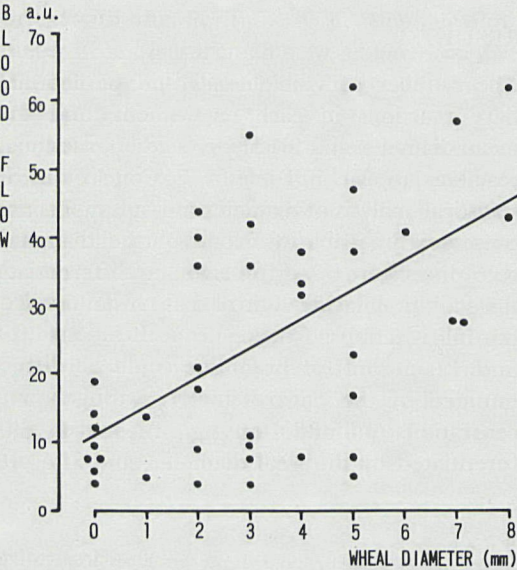


Fig. 2. Relation between cutaneous blood flow and the diameter of the weals as recorded 5 mm from the centre of the weal after 10 min ($r = 0.57$, $P < 0.01$).

min the 0.1 and 1.0 mg/ml concentrations could also be differentiated.

Mean blood flow and SEM in the centre of the weals and 5, 10, 15 and 20 mm from the centre during the experiment are shown in Fig. 1. Differentiation between the reactions and the control was not possible in the oedematous centre of the weal or at the site of the skin-prick, while the better differentiation was obtained 5 and 10 mm from the centre. Conclusions of statistical analysis at those positions are presented in Table 4. Strong (1.0 mg/ml) as well

as stronger (10.0 mg/ml) concentrations could be differentiated by laser Doppler flowmetry, and after 5 min and 5 mm from the centre it was even possible to differentiate the sodium chloride control and the 0.1 mg/ml concentration.

The weal diameter and the blood flow as recorded in the zone of the perilesional flare were correlated (5 mm from the centre, recording after 5 min: correlation coefficient 0.4, $P < 0.05$; 5 mm from the centre, recording after 10 min: correlation coefficient 0.57, $P < 0.01$ – see Fig. 2).

DISCUSSION

Measurement of weal diameter was not very useful for differentiation of weaker reactions (histamine 0.1 and 1.0 mg/ml), except after 10 min. This agrees with the ultrasound study of histamine weals showing that small weals (≤ 5 mm in diameter) expand in thickness, while larger weals (> 5 mm) mainly expand tangentially in the skin (4). Measurement of flare diameter seemed superior to that of weal diameter, however; the former is probably less reproducible in clinical use. In the centre of the weals blood flow measurements showed a similar increase after skin-prick with histamine concentrations as well as sodium chloride. Recordings from the centre of the weal probably reflected the trauma from the skin-prick and the formation of oedema. Flowmetry at the zone of the perilesional flare, however, allowed differentiation between different concentrations of

Table 4
Comparison of laser Doppler blood flow measurements 5 and 10 mm from the centre of the weals as recorded during the experiments – levels of significance

	5 min		10 min		15 min		20 min		30 min	
	5 mm	10 mm	5 mm	10 mm	5 mm	10 mm	5 mm	10 mm	5 mm	10 mm
Sodium chloride control/										
histamine 0.1 mg/ml	0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS
Histamine 0.1 mg/ml/										
1.0 mg/ml	0.05	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05
Histamine 1.0 mg/ml/										
10.0 mg/ml	NS	0.05	0.01	0.05	0.01	0.01	0.05	0.05	0.01	0.01

histamine, as well as the sodium chloride control and, thus, this location was useful for differentiation of the reactions.

Recently, it was found in a laser Doppler flowmetry study of skin reactions after intra-dermal injections of leucotrienes that recordings of the perilesional flare reactions were also suited to quantify these reactions (1). Furthermore, the laser Doppler method was found sufficiently sensitive and reproducible, as compared to the ^{133}Xe washout technique, for quantitation of those reactions (2).

The present study also shows that the time and site of recording should be carefully selected and standardized whenever laser Doppler flowmetry is used to quantify weal reactions. We would suggest flowmetry recordings performed after 10 min and 5 mm from the centre of the weal (the time when measurement of weal diameter is also better suited for quantitation), preceded by flowmetry measurements after 5 min, when differentiation of very weak reactions is possible.

Intra- and inter-individual variations in skin blood flow are known to be significant, and the laser Doppler method is mainly useful for comparative purposes (7, 8). The present study shows that laser Doppler flowmetry is a potentially important new method for quantitative studies of weal reactions. Flowmetry might be useful both in the clinic and for quantifying prick-tests with standard allergens since the increases in flow recorded are quantitatively large. However, a clinically oriented study on flowmetry for quantification of weal reactions in allergic diseases is needed to evaluate the prac-

tical value of this new method. Recently, we have evaluated allergic patch-test reactions by means of laser Doppler flowmetry and ultrasound skin thickness measurements (5, 6). The investigations showed that these methods were useful for quantitation of those reactions as well.

REFERENCES

1. Bisgaard, H., Kristensen, J. & Søndergaard, J.: The effect of leucotrienes C_4 and D_4 on cutaneous blood flow in humans. *Prostaglandins* 23, 797-801, 1982.
2. Bisgaard, H. & Kristensen, J. K.: Quantitation of micro-circulatory blood flow in human cutaneous tissue induced by inflammatory mediators. *J. Invest. Dermatol.* (In press).
3. Malling, H.-J., Andersen, C. E., Boas, M.-B., Holgersen, F., Munch, E. P. & Weeke, B.: The allergy pricker. *Allergy* 37, 563-567, 1982.
4. Serup, J.: Diameters, thickness, area, and volume of skin-prick histamine weals. Measurement of skin thickness by 15 MHz A-mode ultrasound. *Allergy* 39, 359-364, 1984.
5. Serup, J., Staberg, B. & Klemp, B.: Quantification of cutaneous oedema in patch test reactions by measurement of skin thickness with high-frequency pulsed ultrasound. *Contact Dermatitis* 10, 88-93, 1984.
6. Staberg, B., Klemp, B. & Serup, J.: Patch test responses evaluated by cutaneous blood flow measurements. *Arch. Dermatol.* 120, 741-743, 1984.
7. Sundberg, S.: Acute effects and long-term variations in skin blood flow measured with laser Doppler flowmetry. *Scand. J. Clin. Lab. Invest.* 44, 341-345, 1984.
8. Tenland, T.: On laser Doppler flowmetry. Methods and microvascular applications. Linköping Medical Dissertations No. 136. University of Linköping, Linköping, 1982.

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