

Histamine Effect on Human Cutaneous Blood Flow: Regional Variations

ETHEL TUR¹, GALIT AVIRAM¹, DAVID ZELTSER¹, SARAH BRENNER¹ and HOWARD I. MAIBACH²

¹Department of Dermatology, Elias-Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel, and ²Department of Dermatology, University of California School of Medicine, San Francisco, USA

Different reactivities of small blood vessels to the histamine released by exogenous and endogenous substances may play a role in the regional variations of the elicited cutaneous response. To study the regional dependence of cutaneous blood flow in response to histamine, the compound was administered intradermally (prick introduction), thereby bypassing the spatially dependent penetration process. The induced response was quantified with cutaneous blood flow measurements utilizing laser Doppler flowmetry. Extent of response and time parameters were compared. Three anatomical sites, the back, volar side of the forearm, and ankle, were studied on 20 volunteers (10 men and 10 women, age 24–34). For comparison, topical administration was also performed. Significant differences in the measured responses at the three sites were observed: the increase of the cutaneous blood flow on the back was greater than the forearm ($p < 0.01$ prick test, $p < 0.05$ topical application), and that of both sites was greater than the ankle ($p < 0.01$ prick test, $p < 0.05$ topical application). There were no significant differences among the different sites in time parameters and no gender variations. As expected, the time required to reach maximum response was shorter for the intradermal method as compared to the topical application on the back ($p < 0.001$) and forearm ($p < 0.05$). On the other hand, the time required to decrease to 50% of maximum response was not different for the intradermal and topical methods of histamine application. These blood vessel response observations may provide initial insight into inherent functional differences influencing cutaneous manifestations of endogenous and exogenous diseases. **Key word:** Laser Doppler flowmetry.

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E. Tur, Department of Dermatology, Ichilov Medical Center, 6 Weizman St., Tel Aviv, Israel 64239.

Regional variations in cutaneous response to exogenous and endogenous substances have not been extensively investigated. Lahti, in studying benzoic acid contact urticaria, found the upper back and the extensor sides of the upper extremities most sensitive, in terms of intensity of redness response, while the hands exhibited less reactivity, and the soles failed to react (1). Contact urticaria refers to a wheal-and-flare response occurring on the application of chemicals to intact skin, as defined by Maibach & Johnson (2). The development of contact urticaria involves percutaneous absorption of the urticariogenic substance, followed by activation of skin mast cells, immunologic and non-immunologic secretion of histamine (and presumably other mediators), and finally a wheal which results from a direct dilation of and fluid transudation from small dermal blood vessels (3) and a flare reflecting indirect vasodilatation via an axonal reflex (4). Each of the above-mentioned steps may par-

ticipate in the variation of contact urticaria responses among the different anatomical regions (1).

To better understand inherent differences in skin function related to regional variation, we decided to test the hypothesis that different reactivities of small blood vessels via their direct and indirect activation by the released histamine play an important role in the observed regional variation of contact urticaria and perhaps other mediator-related diseases. To bypass the spatially dependent percutaneous absorption process, histamine was intradermally introduced to human volunteers using a dry prick test. The dry skin prick test technique produces reproducible wheal-and-flare reactions, and it is a standardized technique not involving application of solutions onto the skin (5). Three anatomical sites were selected: the back, the forearm, and the ankle. For comparison, histamine was topically applied to the same sites on the contralateral side. Since the histamine-induced activation of the blood vessels affects the blood flow, the reactivity of the cutaneous microvasculature to histamine in the different anatomical sites was quantified with a laser Doppler flowmeter (LDF). This non-invasive optical technique has been used to evaluate the response of cutaneous microvasculature to challenge (5–8). The sensitivity and reproducibility of this method approximate that of the ¹³³Xenon washout technique (9–11).

Thus, this relatively simple in vivo experiment allowed us to objectively compare not only the regional dependence of the reactivity of dermal blood vessels but also the effects of a purely intradermal administration to those of a topical application of histamine. In addition, we compared the results obtained among male and female subjects.

MATERIALS AND METHODS

Subjects

Twenty healthy Caucasian volunteers, ten men and ten women with an age range of 24–34 years, participated in the study. They all gave their informed consent for the study, the protocol of which was approved by the Israeli Ministry of Health Committee for the Conduct of Human Research.

Experimental conditions

Skin blood flow was measured by a commercial LDF (Periflux PF2, Perimed, Sweden), following histamine skin prick test and histamine topical administration. The principles underlying laser Doppler flowmetry have been described elsewhere (9, 10). The depth of penetration of the light in the skin is about 1 mm. The integrator was set at a high value to smooth out fluctuations due to the cardiac cycle. A separate probe holder (PF 104, Perimed, Sweden) was positioned over each site and held in place with double adhesive discs (3M, Minnesota, U.S.A.). As measurements at the center of the wheal are decreased because the extravasated fluid restricts blood supply at the site of histamine administration (12, 13), our measurements were taken 1 cm from the point of histamine administration. For this purpose, a 5-mm hole was drilled

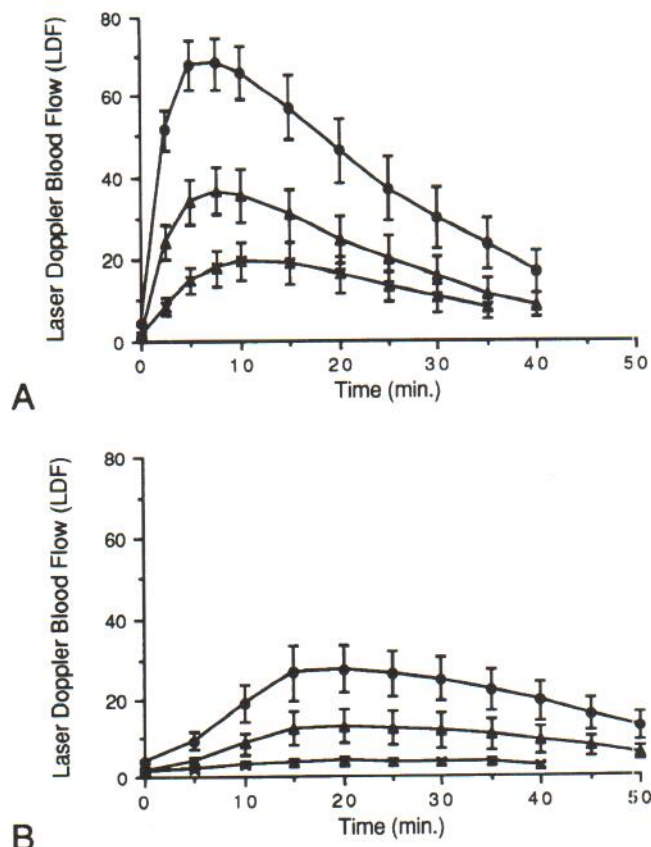


Fig. 1. Cutaneous blood flow as measured by laser Doppler flowmetry as a function of time in the back (circle), forearm (triangle), and ankle (x) following histamine prick test (A) and topical application (B).

in the probe holder at 1 cm distance from the center. This distance appeared to generate the most sensitive and reproducible data according to our prior testing and that of others (6, 13). Taking the measurements at such a distance, and starting them only 2.5 min after the injection avoids the induced transient increase in blood flow, which decays within 2 min (5). After establishing the baseline blood flow at each site, histamine prick test was performed using prick test needles (Phazet™, Pharmacia AB, Uppsala, Sweden), preloaded with histamine chloride (10 mg/ml). The depth of penetration into the skin is 0.79 ± 0.05 (13). To ensure uniformity, all prick tests were applied by the same individual, using a firm pressure for 0.5 s. On symmetrical anatomical sites on the contralateral side, and after taking baseline blood flow readings, 20 microliter of histamine chloride 10% in saline was topically applied by a pipette into the hole (diameter = 5 mm) in the probe holder. The pipette-administered solution remained in contact with the skin surface for 2 min and was then dried gently with tissue. LDF measurements were started immediately and stopped upon reaching 50% of maximum response on the decay phase. Measurements were taken by shifting the probe from one test site to the contralateral site. Each holder was fixed to each test site throughout the experiment, ensuring that all sequential readings were taken from precisely the same location. For each subject, the prick test was randomly assigned either to the left or to the right side, to avoid the controversy concerning the similarity of laser Doppler measurements on bilateral symmetrical skin regions (14, 15). The sequence of measured anatomical sites was random as well. The sites of histamine application were: a) the region of the spine of scapula on the back; b) the ventral surface of the forearm, one third way from the elbow fold to the hand; and c) the medial aspect of the ankle, 5 cm above the medial malleolus. Forearm and the ankle LDF recordings were performed 1 cm proximal to the site of histamine application, whereas in the back they were performed at the same distance medially and diagonally down.

Table I. LDF response following histamine prick test

Values are means \pm SEM for the 20 volunteers. Laser Doppler flowmetry readings are expressed in arbitrary units. For significance see Results.

	Back	Forearm	Ankle
R_{max}	74.4 ± 6.5	40 ± 6.3	21.7 ± 5.2
$A_{1/2}$	1367 ± 271	723 ± 188	423 ± 126
T_{max} (min)	8.8 ± 1.1	8.3 ± 0.8	11.0 ± 1.4
$T_{50\%}$ (min)	22.1 ± 2	18.8 ± 2.5	21 ± 2.5
Baseline	4.4 ± 0.3	1.47 ± 0.2	1.25 ± 0.17

All experiments were performed in the same room under reasonably constant conditions, and during the same season. Subjects rested quietly for 10 min after entering the test room prior to obtaining baseline blood flow readings.

Characteristic parameters

The LDF response to histamine was characterized by four parameters: a) the time required for the response to reach its maximum value (T_{max}); b) the magnitude of the maximum response (R_{max}); c) the area under time-response curve from $t=0$ to $t=T_{max}/2$ ($A_{1/2}$); and d) the time required for the maximum response to decrease to $T_{max}/2$.

Statistics

Comparison between the parameters used to characterize the blood flow response (corrected for the baseline pre-administration value) involved analysis of variance followed by the Newman-Keuls multiple comparison test (16).

RESULTS

Mean LDF response curves for intradermal (prick introduction) and topical applications at each of the three anatomical sites, as a function of time, are shown in Fig. 1. Baseline information, the characteristic response parameters and the results of the statistical analysis appear in Tables I and II. Note that the values described in the graphs are the means at each time point, and since the maximum response was not reached at exactly the same time for all volunteers, the graph reaches a maximum which is lower than the mean of the individual maxima reported in the Tables.

While the baseline of the back was significantly higher than the corresponding baselines of both the forearm and the ankle ($p < 0.01$), the last two baselines did not significantly differ. With intradermal administration of histamine (Table I), the magnitude of the maximum response (R_{max}) as well as the extent of the response as measured by the $A_{1/2}$ were significantly greater on the back than on the forearm ($p < 0.01$ and $p < 0.05$, respec-

Table II. LDF response following topical application of histamine

Values are means \pm SEM for the 20 volunteers. Laser Doppler flowmetry readings are expressed in arbitrary units. For significance see Results.

	Back	Forearm	Ankle
R_{max}	33 ± 7.4	13.3 ± 4.5	4.8 ± 1.6
$A_{1/2}$	723 ± 192	324 ± 148	57 ± 28
T_{max} (min)	19 ± 2	17.3 ± 2.6	17 ± 2.7
$T_{50\%}$ (min)	31.1 ± 3.9	25.4 ± 4.2	17.2 ± 3.6

tively) and the ankle ($p < 0.001$). Furthermore, the R_{\max} and $A_{1/2}$ values of the forearm were greater than those of the ankle ($p < 0.01$ and $p < 0.05$, respectively). There were no significant differences among the three anatomical sites regarding the time to reach maximum response (T_{\max}) and the time required to decrease to 50% of maximum response ($T_{50\%}$).

With topical histamine application (Table II), the results generally exhibited the same order of responsiveness. The R_{\max} value of the back was significantly greater than that of both the forearm ($p < 0.05$) and the ankle ($p < 0.001$). The forearm value was greater than the ankle value ($p < 0.05$). In the extent of the response, as measured by $A_{1/2}$, the ankle value was significantly smaller than that of the back ($p < 0.01$) and the forearm ($p < 0.05$), while the back and the forearm values did not significantly differ. T_{\max} and $T_{50\%}$ did not significantly differ among the three anatomical sites.

A comparison between the intradermal and topical methods of histamine application revealed that the time to reach maximum response (T_{\max}) was significantly shorter on the back ($p < 0.001$) and on the forearm ($p < 0.05$) following intradermal application of histamine. In contrast, the time required for the maximum response to decrease to $T_{\max}/2$ in both methods of application did not significantly differ among the three anatomical sites.

There were no significant differences in any parameter between men and women.

DISCUSSION

Regional variations of the skin response to externally applied chemicals were attributed to differences in: (i) permeability (17, 18); (ii) mast cell density and capacity to synthesize and store histamine; and (iii) concentration of cyclic nucleotide which modulates histamine secretion from mast cells (4). But the intensity of the wheal-and-flare response may be also intimately related to the local reactivity of the blood vessels once the histamine actually reached them, and to their indirect dilatation via the axonal reflex. The histamine prick test of this study bypasses the percutaneous absorption phase, thus enabling a direct investigation of the regional dependence of these final reactions reflected in the changes of the cutaneous blood flow. Laser Doppler flowmetry makes it possible to study both the magnitude of the response and its time course.

The statistical significance of the site-dependence of R_{\max} and $A_{1/2}$, see Table I, indeed suggests that the change in the cutaneous blood flow, as measured by the histamine prick test, is a function of the anatomical site. These regional variations correlate well with the results of Wahlgren & Ekblom (19) who, using a series of histamine injections, visually found a significantly smaller flare area on the forearm than on the upper arm ($p < 0.01$) or on the back ($p < 0.05$).

While the different reactivities of this study were obtained from LDF readings after being corrected for the local baseline, one may still argue that the observed regional dependence of the reactivity is more related to the spatial dependence of blood flow (20) than to variation in the interaction between the injected histamine and the local microvasculature. Note, however, that the similarity between the regional variation of the reac-

tivity and that of the baseline values is not complete in that the baselines at the forearm and ankle did not significantly differ, whereas the reactivity at the forearm site was significantly higher than that at the ankle. We may, therefore, assume that factors other than the basic anatomic structure of the microvasculature of each region, take part in the variance of reactivity among the different regions.

Topical application of histamine was employed by several researchers but contradictory results were obtained in regard to the regional dependence of the response. Shelley & Melton (21), applying 10% histamine topically, did not observe any difference in the degree of urticaria between the sites examined. Cronin & Stoughton (22), in contrast, found the back to be the most responsive site in terms of intensity of erythema and whealing, to 0.01 ml of the base in concentrations of 2%, 5%, 7%, and 10% that was pipetted on the test site. The response at the back was three times that of the peroneal surface of the leg, while the arm reacted with an intermediate intensity. In addition, Lahti (1) found the back most sensitive to topical application of benzoic acid, and the forearm and ankle in decreasing order of responsiveness, in terms of the intensity of redness response. Differences were also noted by Smith et al. (23) who, studying a patient with contact urticaria from cobalt chloride, could elicit an urticarial response only above the waist but not below. While all these results were collected by qualitative visual measurements, our more quantitative LDF-based measurements following topical application of histamine, see Table II, indicate a significantly higher maximum value of response at the back, smaller at the forearm, and even smaller at the ankle. LDF was also used by Magerl et al. (17), who introduced histamine by iontophoresis and obtained small reactions in the forehead, foot and head while the shoulder exhibited a large one. They also demonstrated a proximo-distal gradient of response in both upper and lower limbs.

Regarding the time parameters: the time required for the response to reach its maximum value (T_{\max}) and the time required for the maximum response to decrease to $T_{\max}/2$ ($T_{50\%}$) did not significantly differ among the three anatomical sites in either method of histamine application. The vasodilatory action of the histamine on the human skin blood vessels involves the H_1 and H_2 receptors (4, 24, 25). Activation of either type of receptor can elicit maximum dilation, but the responses differ in their sensitivity to histamine, in the duration of their effect and in the mechanism of their production. H_1 receptors, which reside on endothelial cells, have the higher affinity for histamine and mediate a dilator response that is relatively rapid in onset and short-lived. By contrast, activation of the H_2 receptors, located on vascular smooth muscle cells, causes dilation that develops more slowly and is more sustained (4, 25). In addition, the flare evoked by histamine is a manifestation of an axonal reflex, as it is blocked by local anaesthesia (8). The nerve impulse originates at the site of histamine stimulation, travels centripetally in the cutaneous sensory nerve fibers, and then antidromically down the small branches of the afferent nerves to adjacent arterioles and elicits vasodilatation (26). This action indirectly results in vasodilation which is related to H_1 receptors as well (4). The time similarities as found among the back, forearm, and ankle, as measured by T_{\max} and $T_{50\%}$, may suggest that these three

cutaneous regions possess the same proportions of the two different types of histamine receptors.

The time required for the maximum response to decrease to $T_{\max}/2$ in both topical and intradermal application was not different at the three anatomical sites. Since after carrying out its action the agent is metabolized, independent of the method of its application, our findings indicate that the amount of topically applied histamine was high enough to elicit a response, but not to have a reservoir effect.

Further research is required in order to find the anatomical and functional roots of the variations observed. Possible candidates are the variable density of the cutaneous vasculature, the variance in receptor density and sensitivity, or site-dependence of the density of nerve endings. Hopefully as more is learned about these micro differences in skin function, we will better be able to interpret some of the many localized patterns of skin disease, especially as relates to mediator-related processes.

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