Laser Doppler evaluation of skin reaction in volunteers after histamine iontophoresis

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Abstract

Iontophoresis was used for a non-invasive administration of the agonist histamine. Flares and weals areas were measured after 1%, 0.01% and 0.0001% histamine solution iontophoresis (30 s, 1.4 mA/cm²). There was no clear-cut correlation between area and concentration. 0.0001% histamine solution iontophoresis induced only a vanishing redness. When a typical weal developed (1% and 0.01% histamine), the blood perfusion was lower at histamine administration site between 10 and 40 min as compared to the values recorded during the same time interval in the flare area. When the flare disappeared, the level of laser Doppler flowmetry (LDF) at the weal site still remained higher than basal values. The higher the histamine concentration, the higher the LDF values at flare sites. Controls indicated that only a low and transient increase in LDF values was observed after NaCl iontophoresis (30 s, 1.4 mA/cm²) and that histamine application (1%, 30 s) did not modify basal blood perfusion. Therefore, we suggest to use 1% histamine iontophoresis (30 s, 1.4 mA/cm²) to induce skin reaction to the agonist and to characterize the increase in skin blood perfusion using a laser Doppler velocimeter.

Keywords: Histamine; Iontophoresis; Laser Doppler velocimetry; Flare; Weal

1. Introduction

The evaluation of the activity of antihistaminic drugs in the skin can be assessed by studying their capacity to inhibit the dermal reaction induced by histamine. First there is a local redness, replaced after a few min by a local edema (weal) as histamine increases capillary permeability. At the same time there is activation of chemosensitive unmyelinated afferent nerve fibers. The impulses to the central nervous system occur along with antidromic depolarisation of the nerve endings. Peripheral release of neuropeptides such as substance P contributes to the expansion of the surrounding erythema (flare) [1]. Because percutaneous penetration [2] of topically applied histamine is poor, most investigators are used to perform intradermal injections or prick tests with histamine [3–6]. The skin response is clinically described as a weal and flare reaction. The instrumental evaluation of changes of cutaneous blood perfusion is possible with laser Doppler flowmetry, a highly sensitive method. Nevertheless, injection or prick test produces trauma and disruption of cutaneous tissue which induce a moderate erythema and substantial changes in blood flow dynamics at the prick site and, in its immediate surroundings [5,7]. This is caused by release of vasoactive substances (peptides, histamine) from stimulated nociceptors and mast cells (neurogenic inflammation) [8,9]. Iontophoresis is a
way to promote passage of polar substances through the skin barrier using an electrical potential. Electrophoresis appears to be the major factor of ion migration promotion, in addition to diffusion, electroosmosis and skin permeability enhancement [10]. The ions migrate across the skin without inducing significant skin trauma [10–13]. Taking benefit of the absence of trauma, the weal and flare produced by histamine iontophoresis could therefore be more reliable for quantification of the agonist effects on skin blood perfusion. It has been reported that iontophoretic application of histamine allows quantitative studies of histamine responsiveness with laser Doppler flowmetry [1,14]. Hence, the characterization of skin reaction to histamine iontophoresis in terms of clinical pharmacological modeling has been investigated.

2. Materials and methods

2.1. Volunteers

Eight volunteers (mean age 30 years, range 21–44), with no history of dermatological disease, participated in the study which was approved by the local medical ethic committee on human investigation. The central forearm was used as test site. All volunteers gave their informed written consent to participate in the study and were told that they were free to withdraw from the study at any time.

2.2. Histamine iontophoresis

Preliminary studies were performed with various systems of electrodes and various current densities and durations. The following conditions were selected for histamine iontophoresis. Histamine dihydrochloride (Sigma Chemical Company, St. Louis, MO, USA) was dissolved at 1%, 0.01% and 0.0001% in ultrapure water (Satin 9000, Sation, Barcelona, Spain) or water for injection. The solution of histamine was placed in holes (diameter 6 mm) punched out of hydrocolloid sheets (Duoderm Convatec, Squibb, New Brunswick, USA) applied on the skin. A platinum wire (99.99% purity, Johnson and Matthey, Brussels, Belgium) connected to the positive pole of a Phoresor II device (Iomed, Salt Lake City, UT, USA) was immersed in the histamine solution without touching the skin. Reference electrode was a larger dispersive pad (5 cm × 5 cm) (Iomed, Salt Lake City, UT, USA). Constant current was applied for 30 s at 0.4 mA (1.4 mA/cm²; 12 mC).

Controls were performed without current and iontophoresis (12 mC) was also performed without histamine i.e. with saline solution. Both weals and flares were outlined on translucent paper 10 min after the end of iontophoresis and areas were determined using a graph paper.

To assess the increase in blood flow, a laser Doppler flowmeter (LDF) (Periflux PF3, Sweden) was used. The central parts of five probe holders were assembled as previously described [15]. The central measurement site was overlaid on the histamine application site under visual control and the four other measurement sites were located at a distance of 10 mm. The assembly was correctly fixed with adhesive tape. Measures were performed alternatively at the central site and at the peripheral site which displayed the highest LDF value. The blood flow was monitored for at least 3 h. The results are expressed in PU ± SEM (arbitrary perfusion units; \( n = 5–12 \) data collections per experiment). The final data per minute was the average of five readings made during this minute.

2.3. Statistics

LDF curves were compared by a two-way analysis of variance (Anova) (Scheffe’s F test; \( p < 0.05 \)). Statistical significance of weals and flares sizes differences was assessed using a one way Anova (\( p < 0.05 \)).

3. Results

3.1. Control data

The contact of the skin with histamine solution (1%) for 30 s failed to induce flares and weals and there was no change of basal blood perfusion (4.6 ± 0.8 PU, 10 min after histamine contact). The current application for 30 s at a density of 1.4 mA/cm² on the NaCl solution (0.9%) induced a transient enhancement of about 20 PU. Although, 15 min after NaCl iontophoresis, the LDF readings returned to below 10 PU and no clinically visible modification remained.
Table 1
Weals and flares areas 10 min after histamine iontophoresis (30 s, 1.4 mA/cm²) (mean ± SEM)

<table>
<thead>
<tr>
<th>Histamine concentration</th>
<th>Weal area (mm²)</th>
<th>Weals and flares area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% (n=12)</td>
<td>71.2±5.2</td>
<td>1 255±3 140</td>
</tr>
<tr>
<td>0.01% (n=5)</td>
<td>51.2±24.8</td>
<td>853.2±277.7</td>
</tr>
<tr>
<td>0.0001% (n=7)</td>
<td>Redness 32.0±2.0</td>
<td></td>
</tr>
</tbody>
</table>

*Coefficient of variation (%) = \( \frac{SD}{M} \times 100. \)

3.2. Weals and flares

Flares and weals appeared rapidly after histamine iontophoresis with concentrated agonist solutions (1% and 0.01%). They remained for at least 3 h. The quantitative evaluation of mean weals and flares sizes, 10 min after iontophoresis, shows no differences between two concentrated solutions (Table 1). Nevertheless, iontophoresis of a 0.0001% histamine solution induced only a weak local erythema (without the weal) at the application site in six out of eight volunteers but no redness at the surrounding site was observed except for one volunteer where flare and weal were detected. The coefficients of variation of the measurement of flares and weals area were about 39% and 32% for flares and 25% and 48% for weals for 1% and 0.01% histamine iontophoresis respectively.

3.3. LDF measurements

As compared to baseline values (5 ± 0.4 PU), histamine iontophoresis resulted in an increase of the LDF values, both at the administration site and at 1 cm distance. At the application site of histamine, the LDF values were higher with 1% or 0.01% histamine solutions than 0.0001% histamine solution. After iontophoresis with of 1% and 0.01% solutions, the LDF values progressively increased to a maximum attained about 1 h after iontophoresis. Weals remained pronounced for at least 3 h. However, the LDF values reached the peak at 15 min after 0.0001% histamine iontophoresis and dropped rapidly below 10 PU after 60 min. It represents the weak local redness without edema reversible within 1 h. (Fig. 1). The intraindividual coefficient of variation of LDF measurement 10 min and 60 min after 1% histamine iontophoresis was 29% and 8% respectively (n = 4). The coefficients of variation of LDF measurements, 10 min after histamine iontophoresis, were 20% (1% histamine) and 23% (0.01% histamine) at the site of histamine administration.

The profile of LDF measurements performed at 1 cm from the histamine administration site shows a significant difference between the three histamine concentrations (p<0.05). The higher the histamine concentration, the higher the peak blood flow, and the longer the duration of the vasoactive skin reaction.
phase. The weakest response (0.0001% histamine) was a vanishing increase of LDF readings (less than 15 min duration). The most intense response (1% histamine) peaked between 2 and 45 min and faded away to reach PU values below 10 PU within 120 min (Fig. 2). The coefficients of variation of LDF measurements, 10 min after histamine iontophoresis were 26% (histamine 1%) and 26% (histamine 0.01%) for locations at 1 cm of histamine administration site whereas the intraindividual coefficient of variation was 30% (histamine 1%; n = 4). A characteristic difference between LDF recordings at histamine administration sites and at 1 cm recording sites was a prolonged time period during which increased LDF values were found at the agonist administration site. For each concentration, the LDF values of flares decreased faster than weals values (within 120 min versus more than 180 min respectively) (Fig. 2).

4. Discussion

Clinical signs (weals and flares size) and histamine specific changes of skin cutaneous blood perfusion after histamine iontophoresis at a given current density were investigated. At the site of histamine administration the weals appeared only after the highest histamine concentrations 1% and 0.01%. The lower concentration (0.0001%) induced only a faint and vanishing redness at the application site. The flares were observed when histamine concentration was 0.01% and 1%. The size of weals and flares is subject to a high variability and there was no clear-cut correlation with the concentration. Hemodynamic variables significantly depended on the concentration of histamine solution used for iontophoresis. Moreover, the profile of variation was different at the site of histamine administration as compared with the recordings made at 1 cm distance from the agonist administration site. The LDF values at the histamine administration site needed about 1 h to reach a plateau. When a typical weal developed (1% and 0.01% histamine), the blood perfusion was clearly lower at histamine administration site between 10 and 45 min as compared to the values recorded during the same time interval in the flare area. When the flare gradually disappeared (in general after 60 min) the levels at histamine administration site still remained higher than basal values. Our understanding of this relative dumping of perfusion increase at the histamine administration site has already been proposed with recordings obtained with prick test responses with and without administration of potent anti-H1 agents. Our working hypothesis is as follows: during the first 10 min the extravasation of fluids restricts blood supply at the site of histamine administration due to the high tension in the tissue, which is expanded, and results in a relative decrease of the density of blood vessels, and consequently LDF measurements were quite low [15]. When potent anti-H1 agents were administered, the reduction of this fluid extravasation and the ensuing tension allowed a larger number of red blood cells to flow into the capillary loops at higher speed with as a result increased LDF readings [16]. In the present study, the data are consistent with this view. The coefficient of variation of LDF measurements was better than this of flares and weals area suggesting that the use of hemodynamic parameters to investigate skin reaction to histamine is more adequate. Iontophoresis is a non invasive method to transdermal delivery of drug [11] and control process (1.4 mA/cm², 30 s iontophoresis with NaCl 0.9%) did not induce any flare nor weals. It is an advantage as compared to the significant vascular effects induced by the pricking procedure or the intradermal injection which can be considered as microtrauma. The severity of microinjury and the speed and the volume of injected material are potential factors of variability in measurements made at injection sites [15]. Insertion of the needle or injection of saline produced approximately a sevenfold increase in flow over the base line, the flow increase remained elevated for periods of at least 20 min [7]. As far as LDF is concerned, recording at prick test sites were unable to discriminate between the reactions to saline and agonists. This is partly due to the significant vascular effects induced by the pricking procedure itself [4]. As compared to LDF values after saline iontophoresis (14 PU), saline intradermal injection and drug-free prick test induced higher subcutaneous blood flow (50 and 20 PU, respectively) [15,16]. Therefore, without microtrauma, the skin response to histamine iontophoresis is more specific to agonist effect than after prick test or intradermal injection, even though the coefficients of variation of LDF recordings are similar after the three methods for agonist administration.

Histamine iontophoresis with lower concentration induced only a local redness at the application site. It
was previously reported that soft conditions of histamine iontophoresis (0.16 mC, histamine solution 1%) did not induce acute skin reaction and that flares were observed only in 40% of volunteers [14]. In Heyer's study, LDF data were recorded only at the application site of iontophoresis for 8 min. Nevertheless, in our report, it was shown that the dermal microcirculation was more dependent on histamine iontophoresis conditions around the weal (1 cm from the iontophoresis application site). This observation was also made after skin prick test with histamine [15].

The purpose of this study was to define experimental conditions of histamine iontophoresis inducing pronounced and reversible skin reaction. The inhibitor effect of antihistaminic drugs should be more evaluable with a non invasive and more intense histamine stimulation. Therefore, we suggest to use 1% histamine iontophoresis (30 s, 1.4 mA/cm²) as skin reaction inductor. In such studies, it will be mandatory to state the precise place of measurement and time after challenge when reporting on instrumental evaluation of skin response to histamine [15]. Preliminary studies confirm that histamine iontophoresis is a valuable tool to evaluate the activity of the H1 blocking agents.

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References