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Microvascular response in guinea pig skin to histamine challenge with and without application of skin window

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Summary

We measured the microvascular response to histamine in guinea pig skin. Histamine (40 mg ml^{-1}) was given either as a skin prick test or applied topically onto a skin window. The skin window was prepared by applying suction and gentle warming to the skin so that a blister was formed, and by removing the top of the blister. The microvascular response was measured as the accumulation of radiolabelled transferrin in the skin *in vivo*, reflecting a combination plasma exudation and vasodilatation. In the control (saline) challenge, the response was slightly greater in the skin window than after skin prick challenge and the scatter was larger. Histamine challenge resulted in a significant microvascular response with respect to the control situation when measured immediately after provocation for both challenge techniques. Ten minutes after challenge, a smaller response was measured, which was still significantly greater than control for the skin prick challenge, but not for topical provocation using the skin window technique. We conclude that the microvascular response to histamine after provocation with the skin prick technique is similar to that after topical provocation using the skin window technique. The skin window technique may have a lower sensitivity than the skin prick technique owing to a higher scatter in the control situation. This difference should be considered when performing and interpreting studies of the microvascular reaction in the skin.

Introduction

Inflammatory processes are characterized by a microvascular response comprising vasodilatation and extravasation of plasma. Whereas plasma exudation is considered specific for inflammation, vasodilatation may be produced by inflammatory as well as non-inflammatory stimuli (Persson *et al.*, 1986).

Inflammation in the skin is frequently studied using the skin blister or skin window technique. A skin blister can be formed by applying suction and gentle warming to the skin. The epidermis covering the blister may be removed and a perfusion chamber placed over the exposed dermal surface, and various inflammatory markers, including plasma proteins, can then be measured in the perfusate (Raud *et al.*, 1996). The method has been successful in demonstrating exudative effects of inflammatory stimuli as well as anti-inflammatory effects of various drugs (Gronneberg & Raud, 1996; Gronneberg *et al.*, 1996). However, uncertainty may be held regarding the trauma imposed by skin blister induction. Thus, the concentrations of plasma proteins or plasma tracers in perfusion fluids obtained shortly after the formation of the blister tend to be higher than baseline levels recorded later (Roquet *et al.*, 1995), suggesting some degree of plasma exudative effects by the skin blister induction. By inference from what is known from other tissues, such exudative conditions may be associated with a hyper responsiveness to inflammatory mediators (Greiff *et al.*, 1994, 2003).

We have previously demonstrated that ^{113m}In given i.v. can be monitored in the superficial part of the skin by external detection of its conversion electrons (Karambatsakidou *et al.*, 1996). After i.v. injection of the chloride salt, ^{113m}In forms a stable complex with the plasma protein transferrin (79 600 Da) and therefore changes in microvascular permeability and vasodilatation may be monitored with this technique. Using this technique, we have previously demonstrated the induction of vasodilatation and plasma exudation by histamine prick test challenge and their relationship (Bergh *et al.*, 1996). We have also shown that the skin prick test *per se* induces a slight microvascular response, presumably owing to the mechanical trauma.

The aim of this study was to compare the microvascular response caused by skin blister induction with that of skin prick test and to study the response to histamine challenge using topical application on the skin window and the skin prick test.

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Material and methods

Animal preparation

Forty-eight male guinea pigs, each weighing between 250 and 300 g, were used in the experiments. The animals were shaved on their belly at least 18 h before the experiment. Anaesthesia was induced by administration of a 3:2 mixture of ketamine (Ketalar, 50 mg ml^{-1}) and xylazine (Rompun, 20 mg ml^{-1}) intramuscularly in a dose of 1·0 ml kg^{-1} body weight (Hart *et al.*, 1984). The external jugular vein was exposed and a catheter (PE-50) was introduced trough an incision and secured. The animal was then put on a height-adjustable table under the detector. The animals were kept warm by an infrared lamp. After the measurement the animal was sacrificed by intravenous administration of pentobarbital (Mebumal, 60 mg ml^{-1}) in a dose of 0·2 ml kg^{-1} body weight.

Skin Window technique

The method used in the present study is a modification of the technique described by MacPhee *et al.* (1987). Briefly, a perspex suction chamber with a 3 mm diameter was applied on the shaved belly skin of the animal. Using a suction pump, a negative pressure of about 0·2 kg cm^{-2} was applied for about 50 min until a blister was fully developed. The blister top was removed with a knife.

Radionuclide tracers

We used the radioactive isotope ^{113m}In, which in plasma forms a stable complex with transferrin *in vivo* (Hosain *et al.*, 1969). Approximately 7 MBq ^{113m}In was administered intravenously as an InCl solution, in a volume of 0·5–1·0 ml.

Detector for conversions electrons

To detect the electron emission we used a detector that has previously been described (Bergh *et al.*, 1996; Evilevitch *et al.*, 1999; Karambatsakidou *et al.*, 1996). The output signal was amplified and analysed by a multichannel analyser, which was preset with a 10 s dwell time. The natural background activity was recorded for 2 min before the start of each experiment.

Experimental protocol

There were six experimental groups, each consisting of eight animals. In three groups a skin window was accomplished as described above. In one of these groups (SW_{sal}) a drop of saline was placed on the skin window, left there for 30 s, and then gently removed with a soft tissue. Immediately thereafter ^{113m}In was injected i.v. and the detector was placed over the skin window. In the second group, a drop of a histamine solution (40 mg ml^{-1}) was placed in the skin window for 30 s as described above (SW_{hist}). ^{113m}In was then injected and the measurement started. In the third group, there was an interval of 10 min between the histamine provocation and the injection of ^{113m}In ($SW_{hist+10}$). In the other three groups, skin prick test was performed on intact skin with saline or histamine (40 mg ml^{-1}). In the first two of these groups (SPT_{sal} and SPT_{hist}), the response to saline and histamine was measured immediately and in the third group, there was an interval of 10 min between the histamine provocation and the measurement of the response ($SPT_{hist+10}$).

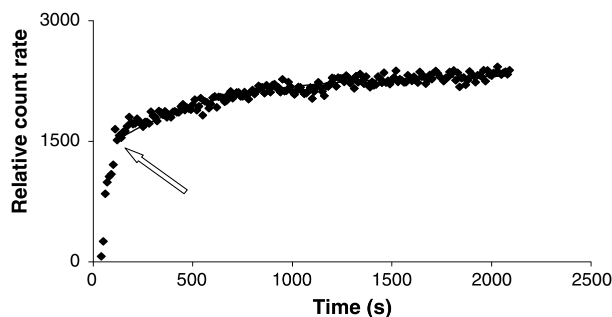

Figure 1 Decay-corrected time-activity curve obtained from an animal experiment. Phase 1 illustrates initial rapid distribution of the tracer in the body and the phase 2 of the curve corresponds to the tracer accumulation during the microvascular response, i.e. vasodilatation and plasma exudation. Arrow indicates the transition from phase 1 to phase 2.

Data analysis

All measurements were corrected for physical decay of ^{113m}In. The time-activity curve obtained from the detector consists of two phases (Fig. 1). The first phase is a rapid rise in count rate corresponding to distribution of the tracer in the body and its arrival into the sampling volume of the detector. The second phase is a slow increase in count rate that gradually levels off into a plateau. The second phase of the curve corresponds to any tracer accumulation by vasodilatation and/or plasma exudation (Bergh *et al.*, 1996; Karambatsakidou *et al.*, 1996). The second phase of the time-activity curve was analysed by fitting the equation

$$C_t = C_1 + C_2[1 - e^{(-kt)}] \quad (1)$$

to the experimental data (Karambatsakidou *et al.*, 1996), where C_t is the count rate at time t, C_1 is the count rate at the end of phase 1 of the time-activity curve, C_2 is the asymptote approached during the microvascular response and k a constant. The magnitude of the microvascular response was expressed as C_2/C_1.

Statistical significance of changes between groups of animals was assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Values of $P<0.05$ were interpreted as significant.

Results

The microvascular response (C_2/C_1) in the different experimental groups is shown in Fig. 2. The ANOVA revealed statistically significant difference between the groups. The

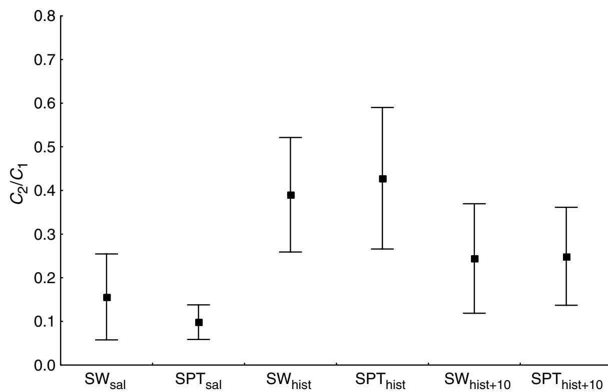

Figure 2 Microvascular response (C_2/C_1) to histamine or NaCl in different experimental groups.

smallest response was seen in the group subjected to skin prick test with saline. A somewhat higher response was seen in the SW_{sal} group, but the difference was not significant ($P = 0{\cdot}33$). However, the scatter was considerably larger in the SW_{sal} than in the SPT_{sal} group, coefficients of variation being 63 and 40%, respectively.

Histamine provocation resulted in similar responses in the SW_{hist} and SPT_{hist} groups. These responses were highly significant compared with groups with saline (SW_{sal} and SPT_{sal}), $P = 0{\cdot}0005$ and $P = 0{\cdot}00003$, respectively. When the measurement started 10 min after histamine provocation, the response was again similar in the $SW_{hist+10}$ and the $SPT_{hist+10}$ groups. The response in the $SW_{hist+10}$ group was not statistically significant compared with control (SW_{sal}; $P = 0{\cdot}14$), but the $SPT_{hist+10}$ group was still significantly different from the control group (SPT_{sal}; $P = 0{\cdot}01$).

Discussion

In the present study, we continue to examine the microvascular response in the skin. This response comprises vasodilatation and plasma exudation (Grega & Adamski, 1988). Whereas vasodilatation is induced by inflammatory as well as non-inflammatory stimuli, plasma exudation, appears to be evoked by inflammatory stimuli only (Grega & Adamski, 1988). Using the present technique, we have previously shown that plasma exudation accounts for approximately two-thirds and vasodilatation for approximately one-third of the response to histamine provocation (Bergh *et al.*, 1996).

Several techniques have been used to monitor the plasma exudation response in the skin. The most frequently employed technique may be the use of the Evans blue dye which after administration intravenously binds to albumin and therefore can be used as plasma tracer (Rawson, 1943). Detection of the dye in the skin can be performed either by planimetry (Allnatt *et al.*, 1990; Elling, 1988) or, more quantitatively, by application of a skin window (Helme *et al.*, 1986; Humphrey & Cavanaugh, 1987; Staberg *et al.*, 1983) or by extraction of the dye from the tissue (Allnatt *et al.*, 1990; Humphrey, 1990; Humphrey & Cavanaugh, 1987). The latter methods are, to some degree, destructive and may themselves have unpredictable effects on the microcirculation in the tissue studied. In the present study, we modified the skin window technique by using radioactive labelling of transferrin and *in-vivo* registration instead of Evans blue dye. Since plasma exudation includes proteins in a wide size range (Greiff *et al.*, 2002), albumin and transferrin would be expected to behave similarly in this model. By radiolabelling with ^{113m}In and detection of conversion electrons, our technique measures the microvascular response approximately 1 mm into the skin (Karambatsakidou *et al.*, 1996). However, the present technique can also be used without the creation of a skin window, in which case it becomes almost atraumatic. Furthermore, parallel determinations of plasma pooling (reflecting plasma exudation and blood pooling) using ^{113m}In-labelled transferrin and blood pooling (reflecting vasodilatation) using ^{111}In-labelled red blood cells can be performed and the plasma exudation response can thus be estimated (Bergh *et al.*, 1996).

Although the skin window technique is certainly less invasive than biopsies, suction and warming of the skin will cause some damage that in itself may give rise to a microvascular response. We therefore wanted to compare the skin window technique to the skin prick test, which we have previously found to cause minimal microvascular response (Bergh *et al.*, 1996; Evilevitch *et al.*, 1999; Karambatsakidou *et al.*, 1996). In agreement with these previous studies (Bergh *et al.*, 1996; Evilevitch *et al.*, 1999), we detected a small microvascular response after skin prick test. The small response in the control situation indicates that the mechanical trauma is minor and the reasonably tight range that the provocation is easy to standardise. In contrast, the response after saline challenge in a skin window tended to be greater than after skin prick test, and the scatter was considerably greater. This probably indicates that the trauma inflicted by the suction procedure is somewhat greater than that of the skin prick test.

When the microvascular response to histamine challenge is measured with the skin prick technique, a graded response is readily demonstrated (Bergh *et al.*, 1996). For this study, we selected a concentration of histamine that produces a moderate degree of plasma exudation (Bergh *et al.*, 1996). The time course of the microvascular response can be followed by injecting the tracer at increasing time interval after the challenge (Evilevitch *et al.*, 1999). In this study, we could measure increased microvascular response immediately after histamine challenge with the skin window technique, but already 10 min later, the response had subsided to the extent that the response was not significantly different from control. A low degree of inflammatory response may therefore be expected to be difficult to detect with the skin window technique.

In inflammatory states, the responsiveness to inflammatory mediator, including histamine, may be increased. For example, in on-going common cold, topical histamine produces abnormally great plasma exudation responses compared with baseline recordings (Greiff *et al.*, 1994). In the present study, no such 'exudative hyperresponsiveness' was detected. The observation

would validate the use of the skin window technique and of topical administrations of histamine as an experimental measure.

In summary, we conclude that the microvascular response to histamine is similar after provocation with the skin prick technique as well as the skin window technique. The skin window technique may have a lower sensitivity than the skin prick technique owing to a higher scatter in the control situation. This difference should be considered when performing and interpreting studies of the microvascular reaction in the skin.

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