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Time course of the inflammatory response to histamine and allergen skin prick test in guinea-pigs

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ABSTRACT

Plasma exudation and vasodilatation are key microvascular features of acute inflammation. Exudation and vasodilatation responses in the weal area after skin prick testing with histamine are essentially completed within 30 min. There is evidence to suggest that vasodilatation lasts considerably longer after provocation with allergen, but there is no information on the duration of plasma exudation. The purpose of this study was to measure the time course of the microvascular inflammatory response in the skin after histamine and allergen provocation. Skin prick tests were performed with histamine, allergen (ovalbumin) or saline (control) on guinea-pigs which were shaved on their backs. Radioactive ^{113m}In was used to label transferrin as a plasma tracer. Radioactivity was recorded from the superficial part of the skin by external detection of conversion electrons from the decay of ^{113m}In. The increase in count rate, corresponding to tracer accumulation by vasodilatation and/or plasma exudation, was used as a measure of the microvascular inflammatory response to skin prick test. The microvascular response was studied immediately and up to 30 min after provocation. The largest response to histamine and allergen occurred immediately after provocation. The exudative response then gradually declined to be absent after 25–30 min. Skin prick test with saline resulted in a small response of shorter duration. We conclude that the microvascular reaction to histamine as well as allergen provocation in guinea-pig skin has a rapid onset and a duration of ≈30 min.

Keywords Beta rays, exudate, inflammation, microcirculation, radioactive tracers

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The immediate microvascular response to inflammatory stimuli comprises vasodilatation and plasma exudation. In the skin, these responses can be elicited by skin prick testing with, e.g. histamine or, in sensitized individuals, allergen (Grega *et al.* 1988). The histamine-induced weal and flare reaction, representing plasma exudation and vasodilatation, is reversible within ≈30 min after challenge (Svensjö & Joyner 1984, Horan *et al.* 1986, Keahey *et al.* 1991). In contrast, the flare reaction after allergen skin prick test in man may last for several hours (Hammarlund *et al.* 1991). No information about the duration of plasma exudation after allergen skin prick test is available.

We have recently described a non-invasive technique which enables the microvascular inflammatory response to be measured in intact skin (Karambatsakidou *et al.* 1996, Bergh *et al.* 1996). The technique is based on external detection of electron radiation from transferrin labelled with ^{113m}In. The electrons have a short range in tissue (≈1 mm) which limits the measurements to the superficial layer of the skin. The purpose of this study was to measure the duration of the inflammatory response in the skin after histamine provocation and to examine any difference in the duration of plasma exudation after provocation with histamine and allergen.

MATERIALS AND METHODS

The study was performed in two parts. In the first, the duration of the inflammatory response after histamine skin prick test was examined in 41 guinea-pigs weighing 250–400 g. In the second, the difference between histamine and allergen provocation was examined in 50 guinea-pigs sensitized to ovalbumin. Sensitization was

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accomplished when the animals were weighing 180–200 g by an intraperitoneal injection of 1 μg ovalbumin and 100 mg $Al(OH)_3$ as adjuvant (Andersson 1980, Erjefält & Persson 1991). The experiment was performed on day 21–28 after sensitization, when the weight of the animal was 350–500 g.

Animal preparation

The animals were shaved on their backs 12 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 through an incision and secured.

Radionuclide tracers

The radioactive isotope ^{113m}In, which after intravenous injection forms a stable complex with transferrin *in vivo* (Hosain *et al.* 1969), was used as a plasma tracer. Approximately 1 MBq ^{113m}In was injected intravenously as InCl solution in a volume of ≈1 mL.

Detector for conversion electrons

We used a recently described technique, which is based on external detection of conversion electrons from ^{113m}In (Karambatsakidou *et al.* 1996, Bergh *et al.* 1996). The electrons have a short range in soft tissue (1.1 mm). Measurement of radioactivity is therefore confined to a superficial layer of the skin and background radiation from surrounding tissues is very low. The detector consists of a plastic scintillator (polystyrene crystal, diameter 6 mm) placed on a photomultiplier tube. The signal was amplified and fed into a multichannel analyser which was preset with a 10-s dwell time. The energy window was set at ±8.8%.

Experimental protocol

Fifteen minutes after the anaesthesia had been induced, the animal was put on a height-adjustable table. The skin prick tests were performed with histamine (40 mg mL^{-1}), ovalbumin (30 mg mL^{-1}) or isotonic saline using a lancet. The lancet was pressed at a 90° angle into the skin through a drop of the solution for 2 s. After 30 s, the solution was wiped off. The detector was then placed immediately over the skin surface and centred on the skin prick test.

In the first part of the study, ^{113m}In was injected immediately, 5, 10, 15 and 25 min after skin prick test with histamine ($n = 6$ in each group) and immediately ($n = 6$) and 5 min ($n = 5$) after skin prick test with saline. Measurement of radioactivity was started immediately before the injection of ^{113m}In. The count rate was recorded for 30 min after the injection of the tracer.

In the second part of the study, ^{113m}In was injected immediately, 15 and 30 min after skin prick test with allergen or histamine in sensitized animals. There were 6–8 animals in each experimental group.

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.

Calculations

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 which gradually levels off into a plateau. The second phase of the curve corresponds to the tracer accumulation during the inflammatory response, i.e. vasodilatation and plasma exudation (Karambatsakidou *et al.* 1996, Bergh *et al.* 1996). The second phase of the time–activity curve was analysed by fitting the equation

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

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 inflammatory response and k a constant. The magnitude of the inflammatory response was expressed as C_2/C_1.

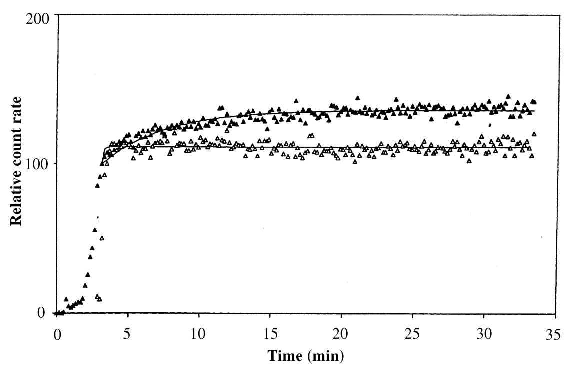

Figure 1 Illustrative time–activity curves after correction for physical decay in a control animal (▲) and in an animal studied with histamine prick test immediately after ^{113m}In-injection (▲). There is an initial rapid rise in count rate reflecting distribution of the tracer. After histamine provocation, there is a second phase of increase in count rate reflecting tracer accumulation by vasodilatation and plasma exudation.

Statistical analysis

Statistical significance of changes between groups of animals was assessed by one-way analysis of variance (ANOVA) followed by LSD test (*t*-test for independent samples). $P < 0.05$ were considered significant. Data are presented as mean ± SEM

RESULTS

Representative time–activity curves obtained when ^{113m}In was injected immediately after provocation with histamine and saline are shown in Fig. 1. The curves were normalized to the count rate achieved after distribution of the tracer (C_1). There was a clear difference in the appearance of the second phase of the curves. After provocation with histamine, there was a further increase in count rate after the distribution phase. A plateau was reached after ≈15 min, and the count rate then remains stable until 30 min after provocation. After provocation with saline, there was little, if any, further increase in count rate after the distribution phase.

The magnitude of the response at different times after histamine provocation is shown in Fig. 2. When the tracer was injected immediately after provocation with histamine, the response was 40% compared with 12% after provocation with saline. When tracer injection was delayed with respect to histamine provocation, the response to histamine gradually declined and was absent 25 min after provocation. ANOVA showed highly significant differences between groups ($P < 0.001$). The LSD test showed significant differences between the control group and tracer injection 5, 10, and 15 but not 25 min after histamine provocation. There were significant differences between the group of animals studied 25 min after histamine provocation and the other groups studied after histamine provocation.

In the animals studied 5 min after skin prick test with saline, there was virtually no change in the count rate after the distribution phase. In two animals, the curve fit resulted in negative values for C_2. In the other three animals, the response was 0.03, 0.05 and 0.06.

The comparison between the inflammatory response to histamine and allergen in sensitized animals is shown in the table. There was very little difference in the response when the tracer was administered immediately after provocation. With delayed tracer injection, the response was only slightly, and not significantly, higher after allergen provocation than after histamine provocation.

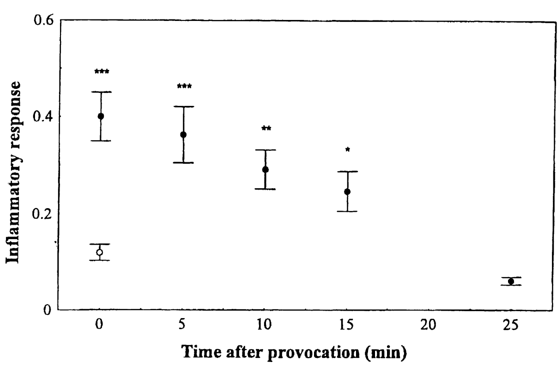

Figure 2 Mean ± SEM of the microvascular inflammatory response in the different experimental groups. (●) indicate animals studied after histamine provocation and (○) animals studied after saline provocation. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. The microvascular response to histamine provocation has a rapid onset and a duration of ≈20 min.

DISCUSSION

In this study, we have used a recently developed technique to measure the time course of the microvascular inflammatory response to histamine and allergen provocation in the skin. The inflammatory response is commonly studied by measurement of the amount of labelled protein present in skin biopsies. This method is destructive and has in itself unpredictable effect on the microcirculation in the tissue studied. Furthermore, there is an obvious risk for contamination with blood. In contrast, the method we used measures the response *in vivo*, with intact circulation in the skin. A further advantage of our technique is that it measures the inflammatory response in a superficial portion of the skin to a well-defined depth. This is determined by the range of the mono-energetic conversion electrons in soft tissue. The short range also means that there is no influence from deeper structures on the measurement.

Intravenously injected ^{113m}In rapidly binds to transferrin, and the radiolabelled protein is then distributed in the plasma volume. The distribution phase gives rise to a rapid rise in count rate over the skin. If no provocation is made, a stable count rate is rapidly reached, reflecting the plasma volume in the sampling volume of the detector (Karambatsakidou *et al.* 1996).

Table 1 Inflammatory response (C_2/C_1) after provocation with histamine and allergen in ovalbumin-sensitized guinea-pigs. There were no significant differences between the two agents at any time

Time after provocation (min)	Histamine	Ovalbumin
0	0.47 ± 0.06	0.48 ± 0.09
15	0.23 ± 0.09	0.34 ± 0.10
30	0.14 ± 0.03	0.16 ± 0.03

After histamine provocation, the distribution phase is followed by a second phase of increase in count rate, reflecting accumulation of radiolabelled transferrin in the skin by vasodilatation and plasma exudation. Lymphatic drainage of the labelled transferrin does not affect the measurement appreciably owing to its slow rate (Staberg *et al.* 1983). In previous studies, we have shown this inflammatory response to be dose dependent after histamine provocation (Karambatsakidou *et al.* 1996, Bergh *et al.* 1996). Vasodilatation accounts for approximately one-third of the response and plasma exudation for approximately two-thirds (Bergh *et al.* 1996).

In the present study, we examined the duration of the microvascular response after histamine and allergen challenge by injecting ^{113m}In at different times after provocation. The rationale is that the second phase of increase in count rate after injection of the tracer will only occur if radiolabelled transferrin is accumulated in the field of view of the detector by vasodilatation and/or plasma exudation. When the tracer was injected immediately after histamine provocation, the inflammatory response resulted in a 40% increase in count rate, confirming previous findings (Karambatsakidou *et al.* 1996, Bergh *et al.* 1996). When the interval between provocation and tracer administration was increased, the inflammatory response gradually declined, and was absent 25 min after provocation. This is in agreement with previous studies performed with other methods (Svensjö & Joyner 1984, Horan *et al.* 1986, Keahey *et al.* 1991).

As in previous studies (Karambatsakidou *et al.* 1996, Bergh *et al.* 1996), a small response was recorded immediately after saline provocation, reflecting a reaction to the mechanical trauma and possibly some bleeding. No response could be detected 5 min after saline provocation, indicating a rapid restitution of the microcirculation after minimal mechanical trauma.

Blood cell flux in human skin after provocation with histamine and allergen has been studied by laser doppler flowmetry by Olsson *et al.* (1988) and Hammarlund *et al.* (1991). After histamine provocation, there is a rapid increase in blood cell flux in the weal area, which subsides during the first hour after provocation. After allergen provocation, on the other hand, blood cell flux remains increased for several hours in the weal area. We found the time course of the inflammatory response to be similar after histamine and allergen provocation. Whereas vasodilatation and plasma exudation thus appear to have similar time courses after histamine provocation, the vasodilatory response seems to have considerably longer duration than the exudative response after allergen provocation. A possible explanation is the wide range of mediators released in response to allergen provocation. Several inflammatory mediators, e.g. prostaglandin D_2 (Heavey *et al.* 1988) and calcitonin gene related peptide (Rogers *et al.* 1988) have, in various rodent tissues, been shown to affect blood flow to a greater extent than plasma exudation.

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