Original paper

Thermographic assessment of skin prick tests in comparison with the routine evaluation methods

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Abstract

Introduction: The skin prick test is still the first and basic procedure in the diagnosis of allergic diseases. The possibility of using a sensitive thermographic method supported by the mathematical model for the assessment of skin test results will be highlighted in the studies.
Aim: To compare the proposed approach with routine planimetric and thermographic methods.
Material and methods: A mathematical model of allergic reaction was developed. Simplifying assumptions of the IgE-mediated skin reaction is the essence of the model. Investigations were performed in a group of 40 patients.
Results: Using the spatio-temporal evolution of temperature distributions, the ratios of the histamine released from mast cells to the control histamine were determined. The obtained values very well correlate with the standard evaluation of skin prick tests (correlation coefficient = 0.98).
Conclusions: The proposed method of skin test evaluation presents several advantages. The continuous acquisition of data provides the monitoring of time course of the allergic response. The transport of mediator and its concentration were distinctly discriminated, which may be diagnostically useful, especially for abnormal cases. The high sensitivity of the method enables studying patients regardless of age and skin sensitivity.

Key words: skin prick tests, thermography, mathematical model.

Introduction

Skin prick tests (SPT) with a routine panel of allergens are still the first and basic procedure in diagnosis of allergic diseases. This is a simple, safe and inexpensive method. The investigation of the skin reaction for allergens allows for treatment planning in the majority of cases. But in some cases, the routine allergy diagnosis can be difficult due to the low or high skin reactivity and other factors. For this reason the new methods of allergic skin response are investigated.

De Weck *et al.* [1, 2] and Bagnato *et al.* [3] reported the use of thermography for skin test evaluation. They used the areas of the heated region and the increase in skin temperature as the diagnostic parameters. Additionally, De Weck introduced so called thermographic unit by multiplying the increase in average temperature by the heated region area [2]. Unfortunately, these thermographic parameters indicate, similarly to the routine method, only the final effect of mediator(s) of the allergic response. Due to the high complexity of the inflammatory reaction, there is no experimental method to assess the direct impact of mediator(s).

Recently, a new method for the assessment of allergen-induced skin reactions has been developed [4]. It was demonstrated that the thermographic measurements supported by the mathematical model, based on the pathophysiology of heat generation, offers a new approach to the quantification of allergen-induced skin reactions. The diagnostically useful information about mediators was obtained.

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Aim

The main objective of this work is to highlight the usefulness of the proposed thermographic procedure for the skin prick test assessment.

Material and methods

Using the information about heat transfer in tissues and the mechanism of the allergic reaction, a mathematical model was developed. The local immune response is a fundamental mechanism considered in the model. This issue is well known and is described by immunology textbooks [5]. The model was described elsewhere [4] and is shown schematically in Figure 1. The simplifying assumptions of the IgE-mediated skin reaction are the essence of the proposed model [4]. These assumptions include only the basic steps of the process. A full description of the local immune response contains a large number of parameters whose values cannot be directly determined from the experimental data.

During the skin prick test, the histamine solution and allergen solutions are introduced into the superficial layers of epidermis. These compounds induce the local immune response. Considering the size of particles and applied time scale it was assumed that the effect is limited to the point of allergen entry while the histamine transport is responsible for the size of the skin lesion. It should be emphasized that the histamine administered as a control and histamine released by the mast cell degranulation are considered separately. The concentration of histamine introduced into the skin (control) was denoted as c_H in Figure 1, while v is the migration rate of histamine. A special role of histamine in the local immune response was confirmed in recent studies [6, 7]. Migrating histamine binds to receptors of nearby capillaries and venules and finally, vasodilation occurs (increase in the diameter of the blood vessel). Vasodilation is a manifestation of a local skin reaction, which changes the temperature distribution of the skin surface. The model solution, under all assumptions, leads to the conclusion that the power generated as a result of increased blood perfusion (an additional heat source) is proportional to the local histamine concentration. Finally, as a result of solution of the heat transfer equation, the increase in the skin temperature (ΔT_H) was obtained [8–10].

The mechanism of skin reaction to the allergen takes place in two steps. In the first step, mast cells are activated by the allergen (Mast cells* in Figure 1). In the second step, the histamine (c_A) is released by the degranulation. It was assumed that histamine is the principal mediator of the allergic reaction. The involvement of other mediators is neglected. Next, the process is similar to the control histamine. The only difference is the concentration of histamine. Finally, the manifestation of the histamine release from mast cells is the increase in the skin temperature (ΔT_A).

Thermographic investigations were performed in a group of 40 patients aged from 18 to 65 years. All studies were performed in accordance with the guidelines of the Jagiellonian University bioethics committee. The studies were performed using the commercial diagnostic allergen panel (Allergopharma, Reinbeck, Germany). According to the routine procedure, a negative control solution and positive control fluid (control solution with 1.7 mg of histamine hydrochloride) were used in the studies. Seven basic inhalant allergens were tested: *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, mixed grasses and mixed trees, mixed weeds, mildews and feathers.

Before the examination, the patients were allowed to adapt for 30 min in order to stabilize the temperature of the skin. After 30 min, the distributions of temperature of both forearms were obtained in order to check the temperature stabilization. A stable temperature of the skin surface was required to initiate further studies.

The skin tests were performed on the palmar surface of the forearms (at least 5 cm from the wrist and 3 cm from the elbow). The forearms of the patient were placed

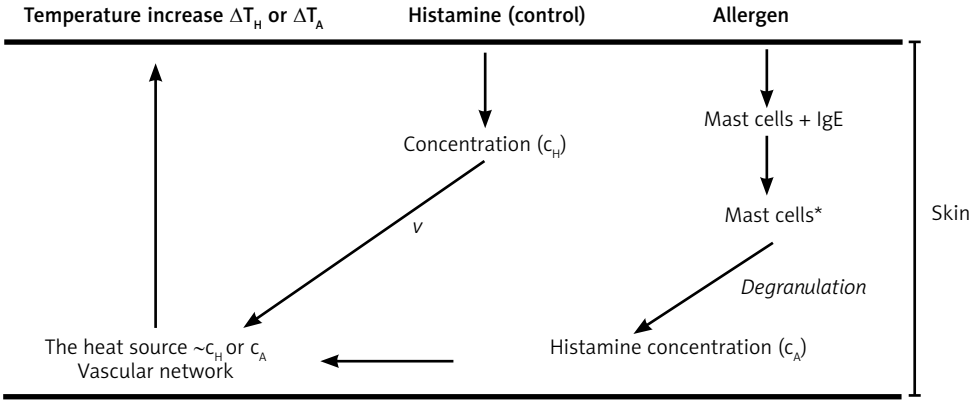

Figure 1. The diagram of the skin allergic reaction model. For details, see the text

on a special table in a position perpendicular to the infrared camera. A thermographic camera (VIGO, Warsaw, Poland) was placed ~30 cm above the forearms. Next, the standard procedure of skin prick testing was performed. A drop of the allergen extract was placed onto the marked area of the skin. Using a sterile lancet, a small prick through the drop was made vertically. Then, a series of thermal images of both forearms were acquired every 70 s. The acquisition time was about 15 min. After 15 min skin responses to all allergens and both controls were evaluated using planimetric measurement by a well experienced technician.

Thermograms were evaluated using the software developed in our laboratories. The first step of the calculations relies on the determination of the temperature increase (ΔT) distributions. For this purpose subtraction of the image acquired before examination from images recorded at different time after allergen introduction was performed. In some cases, a correction for the forearm movement was necessary. The error of ΔT was determined experimentally as equal to 0.3 K with the use of homogenously heated surface [4]. Next, each forearm region heated as the effect of skin response to allergen or positive control was analyzed. The region of elevated temperature was approximated by a circle with a radius *r*. The temperature increase distributions after histamine and allergen injection as a function of time and radius of the heated region were used to determine the model parameters.

Finally, using the mathematical model combined with thermographic measurements, a set of parameters was introduced. The processes of mediator transport and its local skin concentration were significantly discriminated and considered separately by the model. To quantify the allergic response, the concentration ratio of histamine released from mast cells to control histamine was used (c_A/c_H). Also the parameter describing the transport of histamine in the skin layer was considered.

Statistical analysis

Statistical analysis of data was performed using the commercial software Statistica 10 (StatSoft, Poland) at the 95.0% confidence level.

Results

For the studied group of patients, 80 allergic responses were qualified as positive. Regardless of the evaluation method, the negative cases overlapped, so further studies were limited to analysis of the positive responses.

An example of spatio-temporal temperature distribution of the skin surface for histamine injection (positive control) is shown in Figure 2. The presented data were obtained from thermogram analysis which was described in detail above.

An example of spatio-temporal temperature distribution of the skin surface for an allergen is shown in Figure 3. The spatio-temporal temperature distributions for allergens demonstrate similar dependence in shape to histamine control. The only differences were the values of temperature increases and radii of the heated regions.

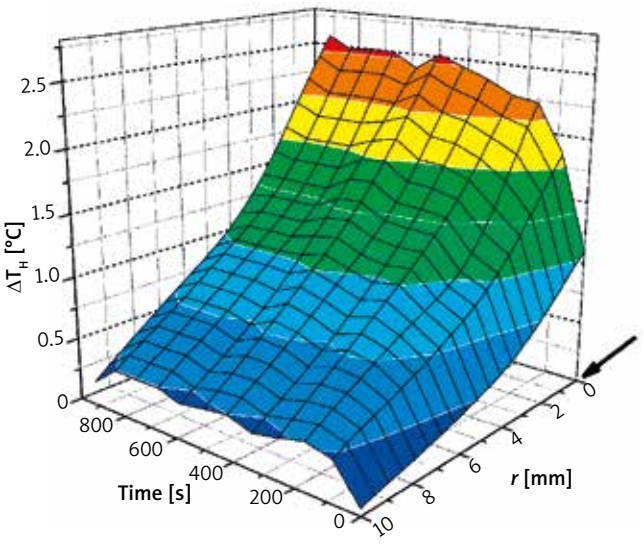

Figure 2. A spatio-temporal temperature distribution for histamine injection (positive control). A point of histamine entry is marked by an arrow, *r* is the radius of the heated area, and ΔT_H is the increase in skin temperature for positive control

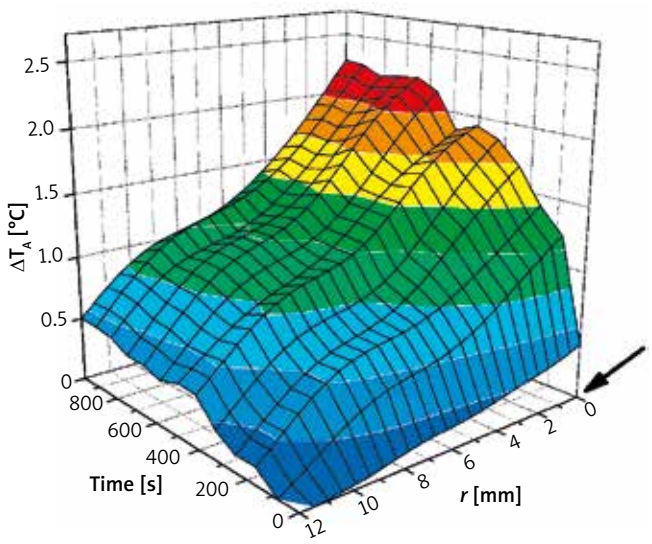

Figure 3. A spatio-temporal temperature distribution calculated from thermograms for the allergen. A point of allergen entry is marked by an arrow, *r* is the radius of the heated area, and ΔT_A is the increase in skin temperature for the allergen

Table 1. Diagnostic data (c_A/c_H) for particular degree of allergic response

Diagnosis	Average	SD	Min.	Max.
(1)	0.40	0.19	0.17	0.69
(2)	0.48	0.21	0.14	0.96
(3)	0.65	0.25	0.40	1.23
(4)	0.84	0.35	0.35	1.35
(5)	0.87	0.22	0.58	1.15

As mentioned above, the diagnostic parameter providing allergic response is a concentration ratio of the histamine released from mast cells c_A to the control histamine c_H. The ratio was directly obtained from fitting of the model solutions. The mean values of the parameter were grouped because of the degree of allergic response in respect to routine diagnosis. The obtained results are shown in Table 1.

The routine standard SPT evaluation method considers a positive response if the wheal's diameter is higher than 3 mm. The following scale was adopted for investigated allergens: (1) – same wheal diameter size as negative control, (2) – induration very small; erythema present = weak reaction (mild), (3) – 50% of wheal diameter size compared to histamine control = moderate sensitivity, (4) – the wheal diameter size same as histamine control = definitely positive, (5) – the wheal diameter size larger than histamine control or with pseudopodia = strongly positive. The grouped results are significantly different from each other ($p = 0.0017$).

Table 2 shows the mean value of diagnostic data (c_A/c_H) for investigated allergens. The allergens were compared between each other. Since the p-value ($p = 0.47$) is greater than 0.05, there is not a statistically significant difference between allergens.

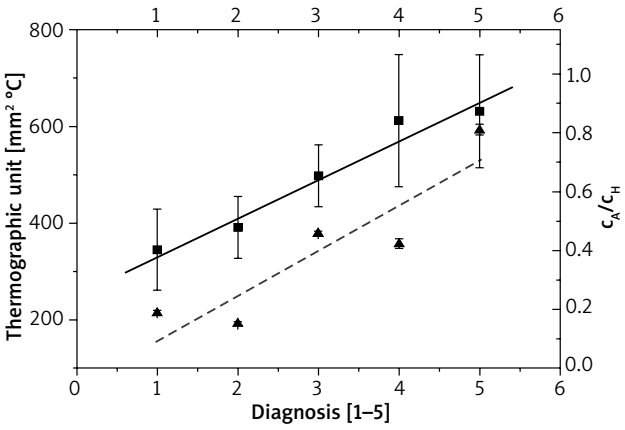

Figure 4. The correlation between the diagnostic parameter (solid lines) obtained from the proposed method (■) and standard thermographic evaluation (▲) with the routine method of skin prick test assessment

Table 2. Diagnostic data (c_A/c_H) for investigated allergens

Allergen	Average	SD	Min.	Max.
D. pteronyssinus	0.65	0.29	0.34	1.35
D. farinae	0.62	0.32	0.18	1.23
Feathers	0.40	0.20	0.14	0.59
Mildews	0.47	0.22	0.17	0.67
Mixed grasses	0.70	0.31	0.27	1.15
Mixed trees	0.66	0.28	0.28	1.19
Mixed weeds	0.48	0.17	0.25	0.72

Discussion

Experiments using the thermographic technique to assess the skin tests, made in recent years [1–3], were limited to determination of the surface of the elevated temperature area size and/or the average temperature increase. Such simple approach still indicates only the final effects of the mediators. The only modification compared to routine methods is the introduction of the infrared digital acquisition of images and digital analysis of lesion, thus the method is more accurate and more sensitive. In order to compare the proposed method and the standard thermographic method, the correlation between diagnostic parameter c_A/c_H and the standard thermographic evaluation [1, 2] with the routine method of skin prick test assessment is shown in Figure 4. The proposed diagnostic parameter better correlates with the standard evaluation of SPT (correlation coefficient $R = 0.98$) than thermographic methods based on temperature and area size evaluation ($R = 0.86$). The results obtained from both methods are also significantly different ($p = 0.013$).

The proposed method is an excellent extension in respect to routinely used methods of SPT evaluation. After developed analysis of the model parameters, it seems that the best one for describing the sensitization is the concentration ratio of the mediator (histamine) released from mast cells and the control histamine. It was used as a diagnostic parameter and its mean values are presented in Table 1. It is important to underline that the routine assessment takes into consideration the mediator concentration and its transport together. In the proposed method these two processes were distinctly discriminated. Moreover, both types of mediator concentrations (the histamine control and histamine released from mast cells) were distinguished by using the spatio-temporal evolution of temperature distributions. The evaluation of SPT is usually limited to one or maximally two points on a time scale, what can lead to false results in some cases. For example, Figure 5 shows the relation between the heated region radius (r) and time after histamine injection for two differently reactive patients. The relation between skin response radius and time acts as in the

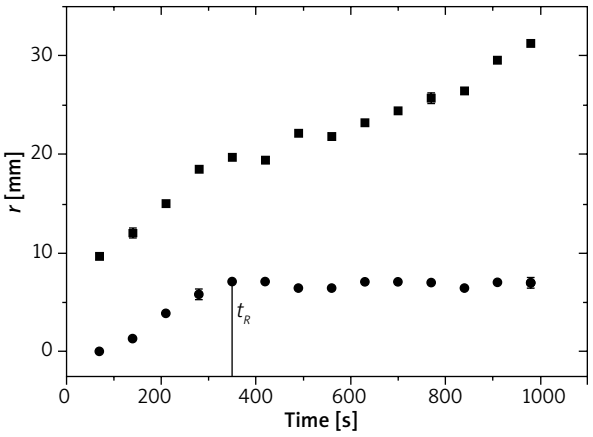

Figure 5. The radius of the heated region versus time after histamine injection for 2 patients

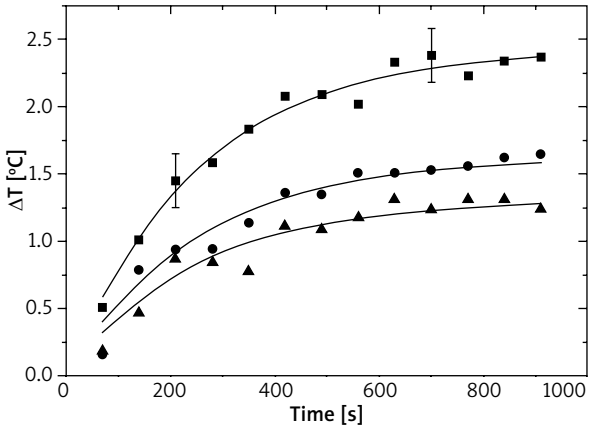

Figure 6. The increase in skin temperature (ΔT) after injection of histamine (■) and allergens (mixed weeds – ● and *D. pteronyssinus* – ▲) versus time for 1 patient. The distance from the histamine injection point is 6 mm in all cases. The determination coefficients were as follows: ■ = 0.98, ● = 0.95, ▲ = 0.93. The solid line presents the fits of the model curves. The error bars marks the experimental errors

case denoted by dots in Figure 5 for most patients. After histamine injection the radius of heated area increases to the specified value *r* at time t_R and achieves the saturation. However, there are cases reacting in a different way (Figure 5 – ■). Such abnormal behavior cannot be discovered by the standard diagnostic procedure. The proposed model approach considers the transport of mediator and its release separately, thus eliminates the source of possible diagnostic errors resulting from diagnosis only based on final effects of the mediators. The parameter describing histamine concentrations plays an important role for these types of cases.

As shown in Table 1 the maximal ranges of mediator concentration ratios do not correlate with the routine method. There is a noticeable larger standard deviation of the diagnostic parameter for a high degree of sensitization (4) than for lower (Figure 4). Because the saturation was not achieved the routine evaluation of skin response can be not exactly correct for these cases. Such underestimation of diagnosis, especially for (4) degree of sensitization, has concerned about 10% of investigated cases.

Table 2 shows the average value of diagnostic data for the investigated allergens. The values of the diagnostic parameter are not significantly different from each other. For some allergens (feathers, mildews and weeds) average values of the parameter are visibly smaller than for others. It is due to a reduced concentration of applied extracts of allergens rather than allergic response or statistics. For these reasons the observed allergic reactions are usually smaller than for other allergens.

Time model analysis of thermograms also provides an opportunity to determine the migration rate of the mediator. Temporal acquisition of control histamine response allows estimating the migration rate of histamine *v* (average *v* = 0.022 ±0.014 mm/s, range = 0.007 ±0.055). The variability range of the parameter can be considered as a confirmation that the immediate allergic response is a highly individual-dependent process. The migration rate of histamine *v* is responsible for the maximal radius of the heated region (*R* = 0.85). This proportionality suggests that the size of the flare is mainly determined by the histamine migration rate. Thus, the determination of the degree of sensitization based on size of the flare seems to be a controversial. Nevertheless, some authors consider a positive reaction if the mean flare diameter is over 10 mm [11]. Further studies are required to unambiguous explanation of the flare usefulness in diagnosis. Investigations with the use of laser Doppler flowmeter can be used for this purpose.

A great advantage of the proposed procedure is its high sensitivity. The small changes of the allergic response can be studied. An example of temporal temperature increase for positive control and two allergens is shown in Figure 6. The degree of sensitization was identified as (2) by the routine diagnosis. The results were fitted with the model solutions (solid lines). Assuming that the concentration of control histamine is equal to 1.0, the concentration of the mediator is as appropriate 0.67 (weeds) and 0.54 (*D. pteronyssinus*). Such assessment cannot be accomplished in the standard evaluation methods.

The proposed method, in some cases, may be treated as an extension of the routine test [12]. In some cases allergy diagnosis can be difficult due to low or high skin reactivity. For example in the elderly patients an issue of correct diagnosis is the subject of recent studies [13]. Many authors claim that skin reactivity to allergens

decreases with age [14]. Photo-damage is also given as a factor causing damages in the skin of elderly and young patients. Sun-damaged skin exhibits both hyper- and hypo-melatonic lesions, accompanied with atrophy of subcutaneous tissue and hypertrophy of the epidermis and increased keratin content [15]. Many researchers consider that current routinely used methods cannot be regarded as a gold standard [16], so the use of the proposed method may prove to be very useful.

Additionally, the great advantage of the proposed method is possibility of determining the error of the diagnostic parameter. It was estimated as ~13%, while in the routinely used methods, the errors are not determined. The skin reaction is considered positive, if the wheal's diameter is higher than 3 mm [17]. This method of assessment can bring about misdiagnosis, especially for a small degree of sensitization. The planimetric measurement of diameter is limited by the precision of the ruler. In case of small wheals, the measurement is encumbered with an error of ~30%.

Conclusions

It was demonstrated that forearm thermography supported by the mathematical model is a noninvasive method to study the allergic response. The spatio-temporal analysis of thermographic images allows for describing the immediate allergic process more specifically. Contrary to the standard diagnostic procedure, which considers only the final stage of the allergic response, the proposed method distinctly discriminates the processes of mediator transport and its concentration. The proposed method, in some cases, may be treated as an extension of the routine SPT. Such advantages as high sensitivity, spatio-temporal possibility of monitoring allergic reaction make this method a supplement to those already existing. Consequently, the proposed method can improve the diagnosis of allergic diseases, and thus leads to improved efficiency of the treatment.

Conflict of interest

The authors declare no conflict of interest.

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