

Laser Doppler perfusion imaging for the documentation of flare-up in contact allergy to gold

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A healed or healing allergic contact dermatitis is easily reactivated to a flare-up if the patient is provoked systemically with his particular allergen. 5 patients with contact allergy to gold were patch tested with a serial dilution of gold sodium thiosulfate. 1 to 2 months later, when positive test reactions were healed, the patients were given 1 intramuscular injection of gold sodium thiomalate. This resulted in a flare-up of patch tests as well as a transient fever and toxicoderma-like reactions. The flaring tests were registered by digital photographs and laser Doppler imaging (LDI). An increased cutaneous blood flow in the formerly positive patch tests was discernible within 1 h after provocation, increased to a vigorous maximum after 4–6 h, and subsided during the following few days. The earliest increase and the highest peak of blood flow were observed in the test areas of the strongest original test concentration. LDI provides a most suitable technique for studying the dynamics of this rapidly developing inflammation of a mainly dermal character.

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Patients with contact allergy demonstrated by patch testing may suffer an exacerbation of their contact dermatitis if they are systemically exposed – accidentally or experimentally – to their contact allergen. With regard to gold allergy, a previous study (1) has shown that parenteral provocation with gold sodium thiomalate (GSTM) may result not only in a flare-up of a previous contact dermatitis and of previously positive patch and intradermal tests, but also in transient fever and toxicoderma-like reactions.

The present study was undertaken to clarify the intensity and time course of the evolving flare-up reactions of healing patch tests to gold sodium thiosulfate (GSTS) following intramuscular injection of GSTM. By and large, the clinical appearance of thus reactivated patch tests is that of a dermal inflammatory process with erythema and oedema (1). Therefore, as an expression of the flare-up, we chose to register the increase of cutaneous blood perfusion by laser Doppler imaging (LDI).

Materials and Methods

Patients, preparation, provocation

5 patients with contact allergy to gold, demon-

strated by patch testing with GSTS (2.0% pet.), were invited to a clinical-experimental study on systemic provocation. There were 4 females and 1 male, with a mean age of 40 years and range 33–66 years. Permission was obtained from the Ethics Committee of the Lund University Medical Faculty and, through informed consent, by the patients.

An aqueous dilution series of GSTS in 9 concentration steps, with a starting-point at 5.0% and with a dilution factor of 10, was applied to the back for 2 days using Finn Chambers[®] on Scanpor[®]. The tests were read on Day (D) 7 using ICDRG criteria for positive patch tests. 1 to 2 months later, the patient was recalled for provocation. In the meantime, the test area, if needed, was delineated with a marking pen.

Before provocation at 9 a.m., the patient was allowed to accommodate in a recumbent position at a room temperature of 23°C. The test area was photographed and the resting cutaneous blood flow was measured by LDI. The patient was then injected intramuscularly with 0.5 ml of GSTM 20 mg/ml (Myocrisin[®] Rhône-Poulenc Rorer, Helsingborg, Sweden). Digital photographs and LDI measurements were then taken repeatedly for 6 h and on following days.

Assay of cutaneous blood flow

Skin perfusion was studied by LDI. In the instrument used (Lisca Development AB, Linköping, Sweden), laser light is directed onto the tissue by means of a system consisting of 2 step motors and mirrors. The light source is a 3mW He-Ne-laser with a wave-length of 632.8 nm. The device is placed 17 cm from the skin surface. In the tissue, light undergoes absorption and scattering. When scattered by moving cells, i.e., erythrocytes and leukocytes, the light undergoes Doppler shift. Some of the backscattered light is detected by remote photo diodes and a signal that scales linearly with the number of Doppler shifted beams and the average Doppler frequency shift is generated for each measured point. A colour coded image of skin perfusion is produced in 64×64 image matrix, representing a 12×12 cm area. Perfusion values are expressed in volts.

In each patient, the patch test area was identified and delineated with black tape, thus enabling it to be traced in the perfusion image. LDI was performed before and 30 min after provocation, and then every hour after provocation. Measurements were also made 1, 3, and 7 days after provocation. At the same times as LDI, digital photographs of the scanned area were obtained (2).

The perfusion images were analyzed by selection of regions of interest corresponding to each patch test site. The regions were approximately 0.9×0.9 cm and were centered on the maximum perfusion value for each test site. A background region was selected over a remote part of the image where the skin had a normal appearance in the photograph. Data were analyzed with, as well as without, a correction for perfusion in the background region.

Results

Clinical reactions

All 5 patients had a transient sense of fever 10–12 h after the GSTM injection; a body temperature of 38–39°C was recorded in 3 of them (Table 1). 1 patient complained of a swollen tongue, lips, and neck as well as numbness in the mouth, arms, and

legs. There were also a few urticarial wheals. In addition, she noticed some itching and exudation of her earlobes. Another patient complained of swollen fingers and itching palms; she also had a discrete maculopapular exanthem.

In all 5 patients, the previously positive but now partially healed patch tests to serial dilutions of GSTS were reactivated. There was an increasing erythema, infiltration, and peripheral oedema, first discernable to the naked eye 2–6 h after the systemic provocation. In all cases, the strongest flare-up occurred at sites where the highest test concentrations had been applied. Here also the earliest start of inflammatory reaction could be observed. In some cases, there was also a flare-up at dilution steps originally considered negative, and at the site of earlier patch tests to GSTS (not to other allergens), now several months old.

LDI

Digital photographs and laser Doppler images recorded 2, 6, and 72 h after injection of GSTM in 1 patient are shown in Fig. 1. The black tape delineating the patch test area is clearly seen in both images. At 2 h, erythema is seen mainly at the sites where high concentrations had been applied in the patch test. This corresponds to sites of increased blood flow in the LDI. At 6 h, the erythema is more intense and widespread. The LDI now shows a large region with increased blood flow in which the patch test sites are discernible as foci of particular intensity. At 72 h, the reaction is subsiding and increased blood flow is mainly seen in the vicinity of the patch test sites.

A quantitative evaluation of the LDI is presented in Fig. 2. Blood flow values obtained in regions corresponding to the patch test sites with the highest concentrations of GSTS and the 3rd dilution step are presented, together with flow values representing the remote background region. There is a dramatic increase in skin blood flow within 1–5 h in all patients at the site of the patch test with the highest GSTS concentration. Blood flow remains elevated at 8 h but again declines at 72 h. At the site of the 3rd dilution step, the increase in

Table 1. Flare-up of patch tests to gold after parenteral provocation: clinical reactions and time course of increased blood flow by LDI

Patient no.	Clinic Signs and symptoms	LDI		
		start	peak	duration
1	fever, itch, urticaria, oedema	90 min	6 h	>24 h
2	fever	2 h	24 h	8 days
3	fever, itch	4 h	6 h	3 days
4	fever	3 h	6 h	7 days
5	fever, itch, rash, oedema	30 min	6 h	7 days

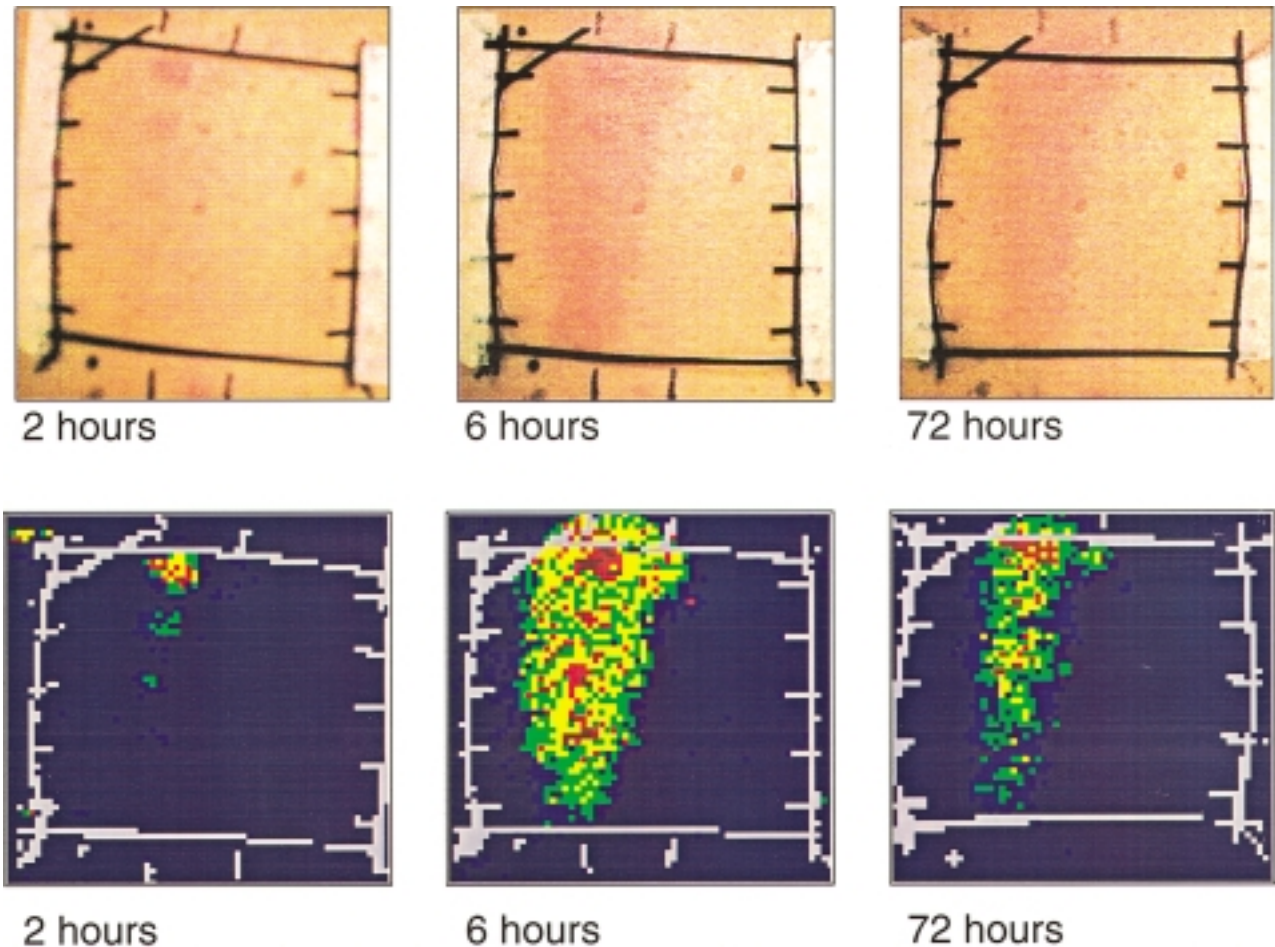


Fig. 1. Digital photographs (top row) and laser Doppler images (bottom row) of the patch test region in 1 patient. The region is delineated by black tape visible in both images. The LDIs show blood flow in a colour scale from blue (low flow) through green and yellow to red (high flow).

blood flow is delayed and the maximum response is lower than at the site of the highest concentration. There is little change in blood flow in the background region.

Discussion

Positive patch tests to GSTS are often delayed with respect to start, peak and end of the inflammatory process (3). Therefore, when retesting our patients with serial GSTS dilution, the tests were not read until D 7. At provocation 1–2 months later, positive tests were still clearly visible and slightly infiltrated. As expected (1), there was a marked flare-up of all previously positive tests after intramuscular injection of a low dose of GSTM (Table 1). In addition, there was an evident reactivation at a few dilution steps originally considered negative. This phenomenon has earlier been demonstrated in contact allergy to gold (1) and to nickel (4). Such falsely negative patch tests have earlier been

shown to feature morphological tissue changes compatible with an allergic reaction (5).

A flare-up was also observed at the site of patch tests to GSTS carried out many months before, not however to other allergens to which the patients happened to be allergic. These results underline the specificity of the present model for systemic provocation with a contact allergen, but may also indicate cross sensitization between GSTS and GSTM.

During the first 24 h after GSTM provocation, there is a marked release in blood of cytokines and acute phase reactants (6). The injected GSTM may also induce a transient fever and general malaise, which might be interpreted as a toxic effect. It has, however, been demonstrated recently (7) that this clinical response is part of the specific allergic reaction during flare-up.

Concomitantly, the flare-up of healed or healing, positive patch tests implies a dramatic change in the histological and immunohistochemical picture,

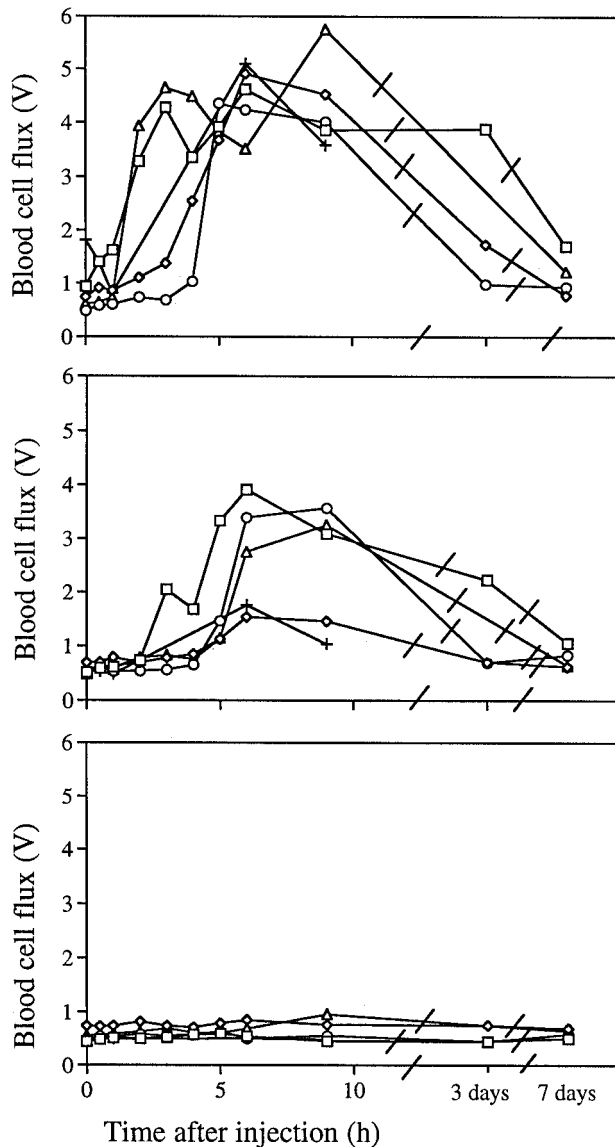


Fig. 2. Blood flow corresponding to the patch test site with the highest concentration (5.0%) of GSTS (top panel), the 3rd dilution step (0.16%, middle panel), and the remote background region (bottom panel). Symbols represent individual patients. Note broken time axis.

with activation of cellular and humoral elements in the epidermis and upper dermis (8). To the naked eye, however, the flare-up seems to be mainly vascularly mediated, featuring intense erythema and oedema as an expression of an increased cutaneous blood flow. By following the flare-up with non-invasive LDI from the resting stage, through the peak, and back to baseline, variables such as intensity and time sequence could be objectively registered.

Laser Doppler techniques have previously been used in contact dermatitis. Thus, by LD velocimetry, Quinn et al. (9) studied experimental in-

flamations elicited by UVB exposure, by a contact allergen and an irritant, as well as by histamine. The effect was assayed on 1 occasion and was found to be correlated to dose. Similar results were obtained in studies on the irritant effect of calcipotriol (10) and of nickel salts (11). A scanning laser Doppler was found most useful when evaluating the blood flow of a skin area induced by a range of doses of a contact allergen (12). Similar conclusions could be drawn when LDI was applied to the heavily increased blood flow in the experimental wheal of cold urticaria (13).

LDI has several favourable features by providing repeated, quantitative measurements of skin blood flow without requiring skin contact or other manipulation. A further advantage in the present study was the type of dermatitis, with its dermal character and minimal disturbance from epidermal damage. This was of course facilitated by the fact that the test areas were not manipulated during the month preceding systemic provocation.

The main LDI findings in the present study were the rapid onset of the vascular reaction at the site of the highest patch test concentration, and a clear relationship between the blood flow response and the patch test concentration. The higher the concentration, the faster the onset of the flare-up reaction and the greater its magnitude. It may be observed that, at maximal flare-up, the reactivated inflammatory areas overlapped (Fig. 1) and thus might have influenced each other. The clear relationship between the strength of the original patch test concentration and the intensity of the flaring test area seems, however, clear (Figs. 1, 2).

Time course studies with LDI on a crescendo dermatitis have been performed with recall allergens of the tuberculin type (14), with the irritant dithranol (15), and with a contact allergen (16). Their results, as well as those in the present study, should be compared with the microvascular effects of mediators of immediate hypersensitivity, such as histamine and bradykinin, which appear within minutes after intradermal injection (17–19). The increase in microvascular permeability observed after histamine or bradykinin injection is completed within 30 min (17, 19) and the increase in blood flow subsides within 1 h (18). LDI, in combination with other techniques such as microdialysis, may provide a suitable means of correlating the microvascular response of the contact dermatitis flare-up with the release of inflammatory mediators in the skin.

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References

1. Möller H, Björkner B, Bruze M. Clinical reactions to systemic provocation with gold sodium thiomalate in patients with contact allergy to gold. *Brit J Dermatol* 1996; 135: 423–427.
2. Bornmyr S, Mårtensson A, Svensson H, Nilsson K-G, Wollmer P. A new device combining laser Doppler perfusion imaging and digital photography. *Clin Physiol* 1996; 16: 535–541.
3. Bruze M, Hedman H, Björkner B, Möller H. The development and course of test reactions to gold sodium thiosulfate. *Contact Dermatitis* 1995; 33: 386–391.
4. Hindsén M. *Clinical and experimental studies in nickel allergy*. Thesis, Malmö, 1998.
5. Möller H, Larsson Å, Björkner B, Bruze M. The histological and immuno-histochemical pattern of positive patch test reactions to gold sodium thiosulfate. *Acta Dermato-venereologica* 1994; 74: 417–423.
6. Möller H, Ohlsson K, Linder C, Björkner B, Bruze M. Cytokines and acute phase reactants during flare up of contact allergy to gold. *Am J Contact Dermatitis* 1998; 9: 15–22.
7. Möller H, Ohlsson K, Linder C, Björkner B, Bruze M. The flare-up reactions after systemic provocation in contact allergy to nickel and gold. *Contact Dermatitis* 1999; 40: 200–204.
8. Larsson Å, Möller H, Björkner B, Bruze B. Morphology of endogenous flare-up reactions in contact allergy to gold. *Acta Dermato-venereologica* 1997; 77: 474–479.
9. Quinn A G, McLelland J, Essex T, Farr P M. Measurement of cutaneous reactions using a scanning laser-Doppler velocimeter. *Brit J Dermatol* 1991; 125: 30–37.
10. Fullerton A, Avnstorp C, Agner T, Dahl J C, Olsen L O, Serup J. Patch test study with calcipotriol ointment in different patient groups, including psoriatic patients with and without adverse dermatitis. *Acta Dermato-venereologica* 1996; 76: 194–202.
11. Wahlberg J E. Nickel: the search for alternative, optimal and non-irritant patch test preparations. Assessment based on laser Doppler flowmetry. *Skin Res Techn* 1996; 2: 136–141.
12. Quinn A G, McLelland J, Essex T, Farr P M. Quantification of contact allergic inflammation: a comparison of existing methods with a scanning laser Doppler velocimeter. *Acta Dermato-venereologica* 1993; 73: 21–25.
13. Andersson T, Wårdell K, Anderson C. Human in vivo cutaneous microdialysis: estimation of histamine release in cold urticaria. *Acta Dermato-venereologica* 1995; 75: 343–347.
14. Stücker M, Auer T, Hoffmann K, Altmeyer P. Two-dimensional blood flow determinations in allergic reactions using laser Doppler scanning. *Contact Dermatitis* 1995; 33: 299–303.
15. Wårdell K, Andersson T, Anderson C. Analysis of laser Doppler perfusion images of experimental irritant skin reactions. *Skin Res Techn* 1996; 2: 149–157.
16. Bjarnason B, Fischer T. Objective assessment of nickel sulfate patch test reactions with laser Doppler perfusion imaging. *Contact Dermatitis* 1998; 39: 112–118.
17. Svensjö E, Joyner W L. The effects of intermittent and continuous stimulation of microvessels in the cheek pouch of hamsters with histamine and bradykinin on the development of venular leaky sites. *Microcirc Endothelium Lymphatics* 1984; 1: 381–396.
18. Olsson P, Hammarlund A, Pipkorn U. Wheal-and flare reactions induced by allergen and histamine: evaluation of blood flow with laser Doppler flowmetry. *J Allergy Clin Immunol* 1988; 82: 291–296.
19. Keahey T M, Indrisano J, White M V, Kaliner M A. Analyses of microvascular permeability in response to mediators of immediate hypersensitivity. *J Allergy Clin Immunol* 1991; 87: 586–594.

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