Acta Derm Venereol (Stockh) 1983; 63: 513–518

Irritation and Staining by Dithranol (Anthralin) and Related Compounds

IV. Visual Estimation of Erythema Compared with Contact Thermometry and Laser Doppler Flowmetry

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Mustakallio, KK, Kolari PJ. Irritation and staining by dithranol (anthralin) and related compounds. IV. Visual estimation of erythema compared with contact thermometry and laser Doppler flowmetry. Acta Derm Venereol (Stockh) 1983; 63: 513–518.

Irritation and staining caused by equi-irritant doses of dithranol (anthralin) and 10-butyryl dithranol (butantrone) were observed for one week after a single 24-hour exposure under occlusion. The test chambers were applied on the uninvolved dorsal skin of 11 psoriasis patients. The estimates of erythema were made with a refined reading scale, and to test the visual discriminatory power they were compared with objective measurements of contact temperature and blood flow of the skin. A sensitive thermistor and a novel laser Doppler flowmeter were used.

On the whole, erythema, contact temperature and superficial blood flow, as interdependent parameters of cutaneous inflammation, all showed a time-dependent statistically significant correlation to the dose of dithranol and 10-butyryl dithranol. The intercorrelations between erythema and blood flow and between erythema and temperature were statistically significant, too. With the doses used, staining of the skin did not hamper the measurement of superficial blood flow, but it did exaggerate visual estimates of erythema at the 4th and 7th day readings. *Key words: Psoriasis; 10-butyryl dithranol; Butantrone; Chamber testing; Erythema; Skin temperature; Skin blood flow.* (Received December 23, 1982.)

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The delayed skin irritation excited by dithranol (anthralin) and related compounds, when sufficiently intense, displays all the cardinal signs of inflammation, namely erythema, increase in temperature, edema, and even pain. When a brownish staining of the skin hampers visual estimation of erythema, contact thermography has been used, but because this method does not give exact numerical values for skin temperature, we decided to compare visual estimates of erythema and staining caused by dithranol and 10-butyryl dithranol, with thermistor measurements of contact temperature. In addition, recent developments in laser Doppler flowmetry (2, 7, 8) have made repeated measurements of blood cell flow at limited skin test areas practicable (9). For the estimation of erythema a refined reading scale was used to compare the visual discriminatory power with thermistor thermometry and laser Doppler flowmetry.

MATERIAL AND METHODS

An unselected series of 11 hospitalized psoriasis patients was tested with 8-mm Finn-chambers using single 24-hour exposures. In previous studies it has been found that the chamber-test method developed by Pirilä (10) is well suited to the estimation of the inflammatory and staining properties of dithranol and related compounds (3, 4), enabling the determination of minimal erythema doses (MED) and irritant doses 50 (ID_{50}) (5, 6). Dithranol (D) and 10-butyryl dithranol (BD) were used as test

compounds and the vehicle white petrolatum served as the control substance. Both testing with an empty chamber and readings on an untreated neighbouring site on uninvolved dorsal skin were used as additional controls. Because D, when expressed on an equimolal basis on the 3rd day, is about 20 times as strong an irritant as BD (5, 6), the test doses were calculated as 'equi-irritant' as possible by using corresponding 3rd day ID_{50} concentrations and their multiples, i.e. 0.5, 1, 2 and 4 ID_{50}. One ID_{50} of D and BD corresponds to 0.005 % and 0.13 % (w/w) concentrations, respectively.

For the visual estimation of erythema, the following more refined reading scale was used in this study, aiming at comparison of the visual discriminatory power with surface thermometry and laser Doppler flowmetry:

0 = no erythema
0.25 = hardly discernible threshold erythema
0.5 = faint ill-defined erythema
1.0 = faint but more defined erythema, about 8–10 mm in diameter
1.5 = precisely measurable slight erythema
2.0 = measurable moderate erythema
2.5 = marked erythema
3.0 = marked intense erythema.

The explanations for the readings 0, 1, 2 and 3 correspond to those of the previous reading scale (3). Even the refined erythema-scale is only relative as regards both the numerical values and also the colour of the neighbour non-erythematous skin site, i.e. 0 reading. The refined erythema scale did not increase the intra-observer error but somewhat enlarged the inter-observer error. It also had a slightly depressive effect on the MED and ID_{50}values. For proper gradation of erythema, the first reactions were read one hour after removal of the test, and re-read 2, 3, 4 and 7 days after application.

The surface temperature was measured at the centre of the test reactions and control sites with an Exacon® thermometer MC8940 using the small, sensitive and rapidly reacting contact thermistor S-2A (Exacon Scientific Instruments, DK-2630 Taastrup, Denmark).

The blood flow at the centre of the test and control sites was recorded with the differential mode laser Doppler flowmeter Periflux® (Perimed, P. O. Box 5607, S-114 86 Stockholm, Sweden). Light from a 2 mW He-Ne laser (632.8 nm) is directed by an optical fibre to the skin surface where laser light penetrates into the skin as a hemisphere with a radius of about 1 mm. A portion of the back-scattered and reflected light is picked up by a pair of fibres, transmitting it to two photodetectors. By the differential detector system and by signal processing, a low noise output signal is obtained, which is proportional to the blood cell flow, i.e. the product (number of erythrocytes moving in measuring volume) × (mean velocity of these cells). Due to the complexity of both the microvascular bed geometry and the interaction of light with tissue, the output signal is expressed in relative blood flow values.

Because both the visual estimation scale and the blood flow values are relative measurements, all the results will be expressed as deviations from the measurements on the neighbouring non-erythematous skin, i.e. as deviations from the 0-level.

Daily dose–response correlation coefficients of erythema, surface temperature and laser Doppler flow and intercorrelations between erythema, surface temperature and laser Doppler flow were calculated. Significance of correlation coefficients was tested by using the *t*-test.

The brownish staining of the skin was graded and read as before (3). Of three independent observers, one measured erythema and staining and the other two temperature or blood flow of the skin.

RESULTS

Control measurements. The control sites, whether non-occluded or occluded, with empty chamber or a chamber filled with white petrolatum, all retained the basis level of skin colour, surface temperature and blood flow.

Visual estimation of erythema. The visual estimates of D and BD erythema during the one-week observation period showed an almost identical course, when the doses of D and BD were calculated as equi-irritant as possible (Fig. 1 *a*). The maximum was reached with all doses of D and BD at the 3rd-day reading.

Contact thermometry. The surface temperature values measured at the centre of the erythema reactions elicited by the equi-irritant doses of D and BD followed, in general, the

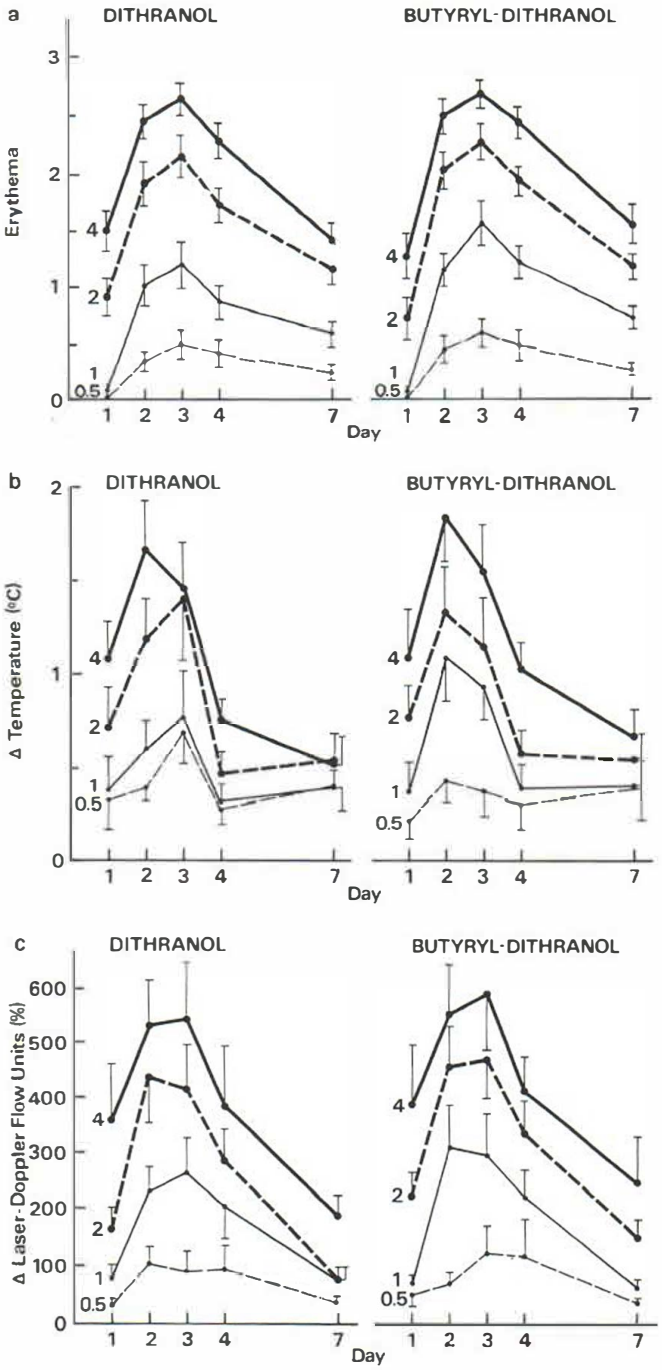

Fig. 1. Means and standard errors of visual estimates of erythema (*a*), surface temperatures (*b*), and laser Doppler flow values (*c*) during a one-week observation of 11 psoriasis patients tested with 0.5, 1, 2 and 4 ID_{50} of dithranol (D) and 10-butyryl dithranol (BD).

course of visual erythema estimates. However, with all doses of BD and with the highest dose of D the maximum surface temperature was reached already at the 2nd-day reading. At the 4th-day reading the temperature values were proportionately lower than the erythema estimates (Fig. 1 *b*).

Laser Doppler flowmetry. The blood flow values followed the course of the visual

estimates of erythema even more closely than the measurements of surface temperature, but also the flow values at the 4th-day reading were proportionately lower than the visual estimates of erythema (Fig. 1 *c*).

Correlations. In daily comparisons, the visual estimation of erythema gave the highest correlation coefficients with the four equi-irritant doses of D and BD, but in general also the other two methods correlate significantly with the doses (Table I). The correlation between visual estimates of erythema and laser Doppler flowmetry were, in general, highly significant (Table II). During the first 4 days surface thermometry was also highly significantly correlated with visual estimation of erythema.

Staining. The brownish staining showed a more delayed course than erythema, being most pronounced first at the 4th-day reading. At the 4 ID_{50} level, none of the patients developed a grade 3 dark brown staining, but 3 patients showed a grade 2 reddish-brown staining to D and one of them also to BD. In general, at equal irritation level BD showed less staining than D.

DISCUSSION

Erythema of pig skin seems to be determined more by the level at which blood circulates than by the blood content of all dermal vessels (1). Also in human skin, possessing superficial capillary loops with cross-shunts, the depth at which the blood circulates

Table I. *Daily calculated linear dose–response correlation coefficients of visual estimation of erythema, surface temperature and laser Doppler flow measurements of skin reactions caused by dithranol (D) and by 10-butyryl dithranol (BD)*

	Visual estimation of erythema		Surface temperature		Laser Doppler flow	
Time	D	BD	D	BD	D	BD
1st day	0.813***	0.747***	0.466**	0.498***	0.603***	0.601***
2nd day	0.803***	0.802***	0.638***	0.623***	0.586***	0.554***
3rd day	0.789***	0.758***	0.386**	0.553***	0.544***	0.547***
4th day	0.823***	0.825***	0.556***	0.632***	0.443**	0.518**
7th day	0.720***	0.736***	0.235	0.318*	0.579***	0.510***

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table II. *Daily calculated intercorrelations of visual estimation of erythema, surface temperature and laser Doppler flow measurements of dithranol (D) and 10-butyryl dithranol (BD) skin test reactions*

	Erythema vs. surface temperature		Erythema vs. laser Doppler flow	
Time	D	BD	D	BD
1st day	0.643***	0.790***	0.548***	0.540***
2nd day	0.848***	0.765***	0.523***	0.631***
3rd day	0.608***	0.694***	0.473**	0.659***
4th day	0.562***	0.631***	0.484**	0.629***
7th day	0.293	0.244	0.586***	0.648***

** $p<0.01$; ** $p<0.001$.

through the skin can be determined, and erythema probably results from a shift of blood closer to the surface of the skin. It is interesting that according to Stüttgen and co-workers (12) the decreased erythema provoked by dithranol in human skin pre-exposed to UVB may be related more to the microcirculation than to a decreased arterial blood flow in the skin, because infrared thermography revealed increased heat radiation in spite of the visually diminished erythema.

As could be expected laser Doppler flowmetry confirmed that the early erythema responses to D and BD were due to increase in superficial blood flow.

The comparatively rapid increase in surface temperature may reflect both metabolic changes in the epidermis and initial inflammation caused by penetrating activated anthrones and simultaneously generated oxygen radicals (cf. 5). 10-butyryl dithranol appeared to cause a faster rise in contact temperature than dithranol, but to be equi-irritant at the 3rd-day reading, 10-butyryl dithranol was used in a concentration 20 times as high as dithranol, and that may cause faster heat production in the epidermis. The stronger and probably deeper involvement of cutaneous vessels on the 2nd and 3rd day caused relatively greater increase in the blood flow values and in the erythema estimates. It should be pointed out, however, that the slowly developing brownish staining, which is slightly stronger with equi-irritant doses of D than BD, hampers the estimation of erythema, especially at the 4th-day reading. This may explain why the visual estimates of erythema at the 4th-day reading were proportionately higher than corresponding measurments of surface temperature and blood flow. In our experience, staining of the skin when reaching the dark brown (grade 3) level disturbs laser Doppler flowmetry, whereas, as such, it does not hamper surface thermometry.

In conclusion, the present study has shown that with the refined erythema reading scale an accuracy comparable to the two more objective methods is attainable.

In the numerical measurement of epicutaneous test reactions laser Doppler flowmetry seems to overcome many difficulties associated with the blood flow methods used so far, including microscopic observations of nail-fold capillaries, clearance of tracers (e.g. xenon), thermal clearance and photoplethysmography (11, 13).

ACKNOWLEDGEMENTS

The authors are grateful to nurses Liisa Kannas and Saga Ylönen for experienced assistance. Mrs Kannas, who since 1978 has estimated dithranol erythema, refined the reading scale.

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