Assessment of skin erythema by eye, laser Doppler flowmeter, spectroradiometer, two-channel erythema meter and Minolta chroma meter

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Abstract. Visual grading, a laser Doppler flowmeter, a spectroradiometer, a two-channel erythema meter and a Minolta chroma meter were compared in the measurement of erythemas arising from immediate contact reactions produced either by 250 mM benzoic acid or 10 mM methyl nicotinate in petrolatum in an open application test or by ultraviolet irradiation. A good correlation between visual grading and objectively measured values was found for all the instruments, but the laser Doppler flowmeter gave proportionally lower values for the ultraviolet erythemas than the skin reflectance meters, suggesting that these differ from erythemas induced by benzoic acid or methyl nicotinate. The laser Doppler flowmeter gave less repeatable results than other meters when measuring moderate and pronounced erythemas produced by ultraviolet irradiation. Measuring both the blood flow and the actual erythema may give more information about the reaction than either measurement system alone.

Materials and methods

Test subjects

The voluntary test subjects were 15 healthy Caucasian medical students, 4 men and 11 women, mean age 25 (20–29) years. They had not received any anti-inflammatory analgesics for 4 days before the tests or had any solarium treatment or sunbathed for the previous 1 month.

Test techniques

A 1 x 1 cm area on the upper back was irradiated with 0.2 J/cm² of UVB in a Waldmann 6002 cubicle with 21 radiation tubes (Philips Ultraviolet-B TL 100 W). About 20 h later, 10-µl doses of the test substances, 250 mM BA and 10 mM MN (both from Sigma Chemical Co., St. Louis, Mo., USA), in petrolatum were applied to 1 x 1 cm areas of the upper back skin at least 10 cm away from the UVB test site and 5 cm apart. After 15 min the test substances were removed by pressing the areas three times for 10 s with a piece of blotting paper.

One reference area for the MN and BA tests was treated with petrolatum alone, and an area of normal untreated skin on the upper back was used as a reference site for the test irradiation with UVB.

Visual grading

All the test sites and reference sites were graded visually. UVB erythema was estimated once, about 20 h after the irradiation and reactions to MN and BA three times, 20, 40 and 60 min after application of the test substances. The following four-point scale for erythema was used: −, none; +, weak; ++, moderate; ++++, pronounced.

After visual grading, the test sites and reference sites were measured with an LDF, a spectroradiometer, a two-channel erythema meter and an Minolta chroma meter. The value for the reference site was subtracted from that for the test site in each case and the difference was used for further calculations.

Key words: Skin colour — Skin blood flow — UVB erythema — Immediate contact reactions — Benzoic acid — Methyl nicotinate

The grading of skin erythema by visual inspection is subjective, being affected by several factors such as viewing geometry, ambient illumination, tanning of the surrounding skin, oedema and the experience and visual acuity of the observer [6, 7]. Therefore, in the quest for reproducible results, laser Doppler flowmeters (LDF), spectroradiometers, spectrophotometers and colorimeters have all been introduced into dermatological research for colour estimation. We report here a comparative study of visual assessment, an LDF and three reflectance erythema meters for measuring erythema reactions caused by ultraviolet-B (UVB) irradiation or by benzoic acid (BA) or methyl nicotinate (MN), two well-known substances eliciting non-immunological immediate contact reactions (NIICRs) in the skin. The main purpose was to find instruments and data which are convenient to use in clinical work and in research.
**Laser Doppler flowmeter**

An LDF is an instrument which detects the flow rate of an object using laser radiation and Doppler shift detection. Thus it measures the total microvascular blood cell flow through the measured volume [5]. The LDF used in this study (PF1, Perimed KB, Stockholm, Sweden) was equipped with a special multifibre probe (Perimed KB, Stockholm, Sweden) which had seven fibre triplets in the probe head, one in the middle and six around it, forming a circle 8 mm in diameter.

**Spectroradiometer**

The broad wavelength distribution of electromagnetic radiation from UV through visible to infrared can be measured using instruments such as spectroradiometers or spectrophotometers. The principal output of these instruments in reflectance measurements is a percentage reflectance curve relative to a calibration object (usually a white reference surface). These measured spectral curves provided the raw data for spectral analyses, ingredient identification or the computation of tristimulus colour values. For this study we used a commercial spectroradiometer system, Rofin Sinar RSO 6230 spectral Processor, RSO 6000 Optical Spectrum Analyzer and RSO 6160 Light Source (Rofin Sinar Laser UK, Weybridge, UK). The instrument was equipped with a fibre-optic sensor probe of diameter 8 mm for reflectance measurements.

The emission from the light source is fed through the fibre-optic probe to the test site at an angle of 45° and the probe collects the reflected light in the same 45° geometry (diffuse reflection) and feeds it into the input slit of the optical spectrum analyser. A rotating diffraction grating then scans the wavelength range and the processor analyses this data and displays the result on an integral monitor or prints it out on paper. The instrument must be calibrated against a white reference substance (barium sulphate) or some other object specified by the user before measurement.

In the measurement procedure the fibre-optic probe was located at a 45° angle to the skin surface. The measurement sequence took roughly 2 min (including averaging of 100 measurement cycles, calculations and storage of the result file on a floppy disk). The measured result could be displayed as a spectral reflectance curve relative to the reference or as CIE (Commission Internationale de l’Eclairage) colour coordinates. Further analyses were performed using a relative reflectance index, the relationship between two wavelengths, one in the blood/hae-moglobin absorption band (555 nm, bandwidth 30 nm) and the other in a reference band (660 nm, bandwidth 30 nm). The instrument consists of a fibre-optic sensor head, a microprocessor-based control and analysis unit and a plotter. The relative reflectance program developed for use with the instrument calculates, displays and prints out the ratio $R_1$ (555 nm) to $R_2$ (660 nm) as the measurement result. The sensor head can be equipped with two mechanical parts to yield the optimal measurement distances with a 45° or perpendicular (0°) geometry. The sampling area on the surface of the skin is 7 mm in diameter [9].

In a typical measurement procedure the instrument was first calibrated automatically against a reference surface (white standard or reference skin site); this took 7 s. The program started by plotting a header, after which the instrument was ready to perform measurements. After positioning the sensor head on the skin surface, the measurement function was activated with a hand-held switch. Measurement took 2.5 s (averaging 4096 individual measurements) and the result was plotted immediately. A new measurement could be started while plotting was still in progress, providing a measurement sequence of about 5 s.

**Minolta chroma meter**

The Minolta chroma meter, and other tristimulus colorimeters, are instruments that have source–filter–photodetector combinations, that simulate the Standard Observer functions, and a unit that directly computes the chromatic dimensions of the colour. The accuracy of the instrument for colour measurements is limited by the fit of the Standard Observer curves and the real filter responses [12]. A Minolta croma meter Type CR-200 (Minolta, Osaka, Japan) was used in this study. The measurement mode used was $L^*a^*b^*$, and the $a^*$-value (red–green axis) was used for further calculations [14].

A detailed description of the equipment is published elsewhere [12]. The results were analysed statistically using Student’s $t$-test, the $t$-test for paired observations, and a one way analysis of variance with Bonferroni a post priori test, and Spearman’s rank order correlation coefficients were calculated.

**In vivo repeatability of the measurements**

UV erythema was produced with four UV lamps (Osram Ultra-Vitalux, 300 W, Italy) mounted 7 cm apart. One healthy Caucasian male (MH, one of the authors) volunteered for this part of the work. Squares on the upper back (1 x 1 cm) were irradiated with increasing doses from 10 s to 140 s, in 10-s steps.

For the comparison of the visual evaluations with the four erythema meters, the squares with a minimal erythema dose (MED) and that with 4 MEDs were chosen for evaluation, with a non-irradiated area of skin as the reference site. A total of 30 successive measurements were performed 20–22 h after UV irradiation. The subject was in a sitting position at a room temperature of 23 °C, and the instruments were used in the following order: LDF, spectroradiometer, two-channel erythema meter and Minolta chroma meter.

The dynamic range of each instrument was calculated as follows: $X_n - X_n$, where $X_n$ the mean of the 30 measurements from the 4-MED test site and $X_n$ the mean of the measurements from the unirradiated normal skin. The standard deviation (SD) represents the

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**Two-channel erythema meter**

The fibre-optic two-channel erythema meter was built in the Electronics Laboratory, University of Oulu, Finland [9]. It measures the reflectance of the skin at two wavelengths, one in the blood/hae-moglobin absorption band (555 nm, bandwidth 30 nm) and the other in a reference band (660 nm, bandwidth 30 nm). The instrument consists of a fibre-optic sensor head, a microprocessor-based control and analysis unit and a plotter. The relative reflectance program developed for use with the instrument calculates, displays and prints out the ratio $R_1$ (555 nm) to $R_2$ (660 nm) as the measurement result. The sensor head can be equipped with two mechanical parts to yield the optimal measurement distances with a 45° or perpendicular (0°) geometry. The sampling area on the surface of the skin is 7 mm in diameter [9].

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**Fig. 1a, b. Comparison of laser Doppler flowmeter (LDF), spectroradiometer (SR), two-channel erythema meter (2-C), and Minolta chroma meter (MCM) measurements of skin erythema produced by 10 mM methyl nicotinate after 20 min a and by 0.2 J of UVB after 20 h. b. The mean of the reactions to 250 mM benzoic acid at 20 min was used as a positive reference and set at 100. The differences between the values shown in a are not significant. In b LDF/SR, p < 0.05; LDF/MCM, p < 0.01; other differences are not significant (Student’s $t$-test)**