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Histological Examination in Assessment of Ultraviolet-Induced Suppression of Contact Hypersensitivity Response*

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The evaluation of contact hypersensitivity (CHS) reaction is one of the methods used in the assessment of the immune status of an organism after UV radiation. The aim of the study was to compare usefulness of visual scoring system and histological morphometry in the assessment of CHS response after exposure of humans to solar simulated radiation (SSR). The study included 140 healthy volunteers, 33 people were irradiated for 2 days, 34 – for 10 days and 33 – for 30 days with SSR. Forty non-irradiated individuals served as controls. All the volunteers were sensitized with diphenylocyclopropenone (DPCP) 24 h after final exposure. Statistical analysis comparing intensity of CHS reaction based on visual score between irradiated groups and non-irradiated group revealed no differences ($p>0.05$). We found a significant difference in epidermal thickness between healthy skin and irradiated groups ($p<0.05$) and a positive correlation between intensity of spongiosis and clinical score for CHS response at 3.2 DPCP site ($p<0.000001$). A negative correlation between time of irradiation and spongiosis score was revealed ($R=-0.28$; $p<0.001$). We conclude that histological examination of biopsies taken from one of the series of elicitation sites is a reliable and sensitive method in the evaluation of CHS response after UVR.

Introduction

Solar ultraviolet radiation (UVR) is one of the most important environmental factors which may cause sunburn, erythema and ocular damage [5, 6, 12]. UVR is also involved in cancerogenesis, impairment of resistance to infections and influences cutaneous and systemic immunity [1, 10]. It is proven that moderate and high doses of both UVB and UVA radiations lead to immunosuppression.

The evaluation of contact hypersensitivity (CHS) reaction is one of the methods used in the assessment of the organism immune status. CHS is a very reliable and informative measure of cell-mediated immune function that is partially dependent on UV radiation.

The CHS reaction depends on the generation of a delayed T-cell response to epicutaneously applied sensitizing antigens or haptens e.g. diphenylocyclopropenone (DPCP). Specifically primed T lymphocytes from the first contact are capable of recognizing the same hapten at a later date and conduct powerful inflammatory reaction. Allergic response under physiological condition appears after 48 hours when some weeks later a subject is challenged by second exposure to different hapten titrations. In photobiology most human studies are performed in healthy volunteers, so that in clinical practice the intensity of elicitation is mostly determined by visual scoring system [7, 8, 11]. Histological examination may also bring many details on the intensity of CHS response however it is not often used in humans because of ethical issues such as necessity of biopsy taking.

The aim of our study was to compare two methods: visual scoring system and histological morphometry in the assessment of CHS response after UVR exposure.

Material and Methods

Subjects

The study group consisted of 140 healthy volunteers aged 19–51 (median 25.5 years), who were not previously

*This study was supported by the European Union research project number QTL-CT-2001-00212IHA-UV and Medical University in Łódź research project number 503-119-1

exposed to contact allergens (DPCP). Each volunteer gave written informed consent before entry into the study, and the experimental plan was approved by the local Ethics Committee.

One hundred individuals were irradiated (whole body irradiation in two half-walls cabinets) and divided into three groups. Thirty three people (17 women, 16 men; mean age 26.1 years) were irradiated for 2 consecutive days (2-day group), 34 (13 women, 21 men; mean age 24.3 years) – for 10 consecutive days (10-day group) and 33 (16 women, 17 men; mean age 26.7 years) for 30 consecutive days (30-day group) by solar simulated radiation (SSR) with a daily dose of 1.2 SED (standard erythema dose when 1 SED is equivalent to an erythemal radial exposure of 100 J/m^2) [4].

Additionally 40 non-irradiated individuals (17 women, 23 men; mean age 25) were assessed as a control group. All the subjects from four groups were sensitized using DPCP (protocol described below).

For histological analysis we also designed another 25 individual group, age and sex matched (12 women, 13 men, mean age 25.4) who were neither irradiated nor sensitized. The skin samples were taken from the same body site as in the other groups and served as control for the histological examination.

UV irradiation

The SSR was generated by 100 W Cleo *Natural* lamps (Philips, Eindhoven, the Netherlands) giving an even field of irradiance (4% UVB 280–315 nm; 96% UVA 315–400 nm) of about 4.95 mW/cm^2 (280–400 nm) on the skin surface at the distance of 20 cm from the source. Measurement of the intensity of the Cleo lamps was performed with the Solar Light 3D UV meter (Solar Light co., Philadelphia, USA).

Hapten, sensitization and elicitation

DPCP was obtained from Fluka Chemie GmbH, Buchs, Switzerland. The hapten solutions were made up freshly before use.

For sensitization 50 mg of DPCP was diluted to 50 ml in acetone (20 μg/20 μl). The subjects were sensitized on left irradiated or non-irradiated (control group) buttock skin using two 7 mm petrolatum-backed filter discs, one soaked in 20 μl of 0.1% DPCP. The irradiated subjects were sensitized 24 h after a final exposure to SSR. The filters were mounted inside 8 mm aluminum Finn chambers (Epitest Ltd, Tuusula, Finland) and two chambers were taped to the skin with hypo-allergenic scanpore tape and left for 48 h. Volunteers were requested to keep the patch dry for 48 h, after which the patch was removed. For elicitation three weeks later all the volunteers from irradiated groups received an antigenic challenge on unirradiated upper inner left arm skin with titration series of DPCP. Petrolatum-backed 7 mm filter discs were placed in 8 mm aluminum Finn chambers soaked with 20 μl of hapten solution of various strengths. The patches contained incremental doses of DPCP: 0.4 μg (20 μl of 20 μg of DPCP in 1 ml of acetone), 0.8 μg (20 μl of 40 μg of DPCP in 1 ml of acetone), 1.6 μg (20 μl of 80 μg of DPCP in 1 ml of acetone), 3.2 μg (20 μl of 160 μg of DPCP in 1 ml of acetone). One patch was only soaked with acetone control. Five patches were placed on the left arm and remained there for 6 h. The elicitation sites were marked on each arm with surgical marker. At 48 h elicitation sites were clinically evaluated.

Histological examination

A 3 mm-punch skin biopsy was taken from the sensitized by 3.2 μg of DPCP site in each of the challenged subjects. Additionally, 3 mm punch skin biopsies were taken from 25 non-sensitized, non-irradiated volunteers. The tissue specimens were fixed in 10% buffered formaldehyde and routinely processed for paraffin embedding and cut. Then the sections were dewaxed and subjected to typical hematoxylin and eosin staining. Sections 3–4 μm thick were cut. The skin specimens were evaluated by two independent pathologists (400× high power fields Olympus Bx system microscope in ten sequences).

Histological morphometry was performed by means of an image analysis system consisting of IBM-compatible computer equipped with an optical mouse, Indeo Fast card (frame grabber, true-colour, real-time), produced by Indeco (Taiwan) and colour TV camera Panasonic (Japan) linked to a Carl Zeiss Jeneval microscope (Germany). This system was programmed (program MultiScan 8.08, Computer Scanning system, Poland) to calculate the intensity of spongiosis in the whole specimen and the minimum, maximum and mean thickness of the epidermis in the whole specimen.

In each group of the volunteers histological analysis included the assessment of the following parameters: total thickness of the epidermis (mean value obtained from 10 sequences) and intensity of spongiosis (0 – no spongiosis, 1 – slight intracellular edema, no intraepidermal vesicles, 2 – edema and single intraepidermal vesicles, 3 – severe edema and multiple intraepidermal vesicles) calculated from 10 sequences.

All obtained results were compared between sensitized-irradiated and sensitized-unirradiated individuals. The same analyzed parameters were measured in healthy, non-irradiated-non-sensitized volunteers.

Statistical analysis

Statistical analysis of data was performed using Mann-Whitney test, Wilcoxon pair test, chi^2 test and non parametric correlation of Spearman. A p value less than 0.05 was considered as statistically significant.

Results

Characteristics of volunteers from irradiated groups and controls are shown in Table 1.

TABLE 1
Characteristics of the volunteers

No of group	No of subjects	Mean age years	Gender F/M	Mean MED J/cm^2	Phototype II/III
Control	40	25	17/23	0.18	19/21
2-day group	33	26.1	17/16	0.16	17/16
10-day group	34	24.3	13/21	0.16	22/12
30-day group	33	26.7	16/17	0.15	22/11

Visual assessment of allergic responses

The intensity of contact hypersensitivity elicitation response was visually assessed using subjective scoring system: (0) = no reaction, (1) = macular erythema, (2) = erythema with infiltration, (3) = erythema with infiltration and papules or vesicles, (4) = bullous reaction. In the control group, 36 individuals (78.5%) were successfully sensitized with DPCP at the lowest concentration of DPCP, and 90% – at the strongest one (3.2 μg). Blistering reaction was observed in 1 volunteer (2.5%) at 0.4 μg DPCP site and in 6 volunteers (15%) at 3.2 μg DPCP one. In the 2-, 10- and 30-day groups the number of non-sensitized subjects at the lowest concentration of DPCP was: 8 (24.2%), 12 (35.3), 18 (54.5%), respectively. At the strongest titration of DPCP no reaction was observed in 5 subjects (15.1%) in the 2-day group, in 4 (11.8%) in the 10-day group and in 9 volunteers (27.3%) in the 30-day group. Detailed results of CHS response at 3.2 DPCP site in all the volunteers are demonstrated in Figure 1.

Statistical analysis comparing intensity of CHS reaction, based on visual score, between irradiated groups: 2-day, 10-day or 30-day and non-irradiated group revealed no differences ($p>0.05$). Sum clinical score (result obtained from all DPCP concentrations in all volunteers) from UV-exposed and non-exposed groups and its comparison with time of irradiation showed no correlation ($p>0.05$).

Histological analysis

In sensitized and unirradiated individuals (control group) the mean thickness of the epidermis was 0.207 mm, which was significantly higher than in the completely healthy skin (non-sensitized, non-irradiated) (mean value 0.061). In a 2-day irradiated group the mean thickness of the epidermis was: 0.166 mm, in a 10-day: 0.163 mm and in a 30-day: 0.108 mm. We found a significant difference in epidermal thickness between control group and healthy skin, and between the latter one and irradiated groups ($p<0.05$). There was a tendency to negative correlation between mean thickness of the epidermis and time of irradiation but the difference did not reach statistical significance ($p>0.05$). The mean thickness of epidermis correlated positively with the sum score of CHS response in all the groups ($p<0.05$).

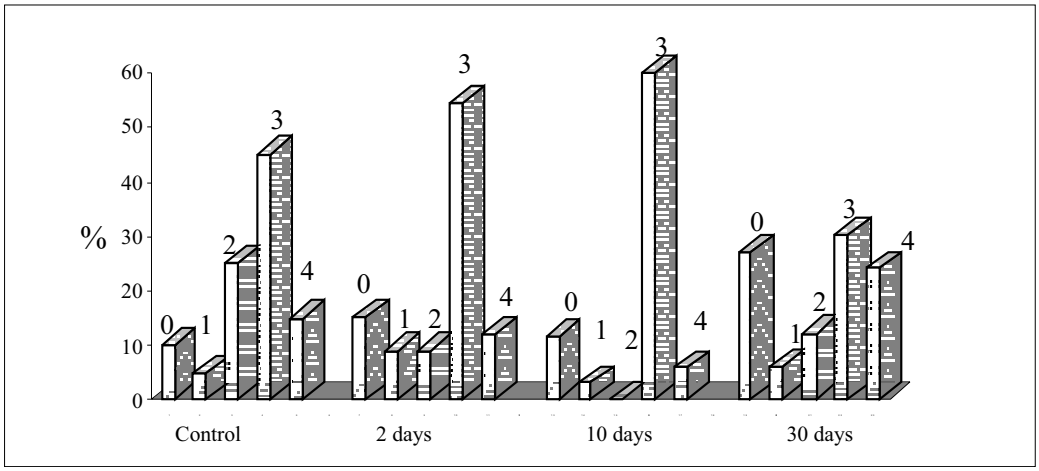

Fig. 1. Frequency of occurrence of CHS response at 3.2 DPCP site in all the volunteers: a) Controls (group 4) n=40
0 = no reaction; 1 = macular erythema; 2 = erythema with infiltration; 3 = erythema with infiltration and papules or vesicles; 4 = bullous reaction.

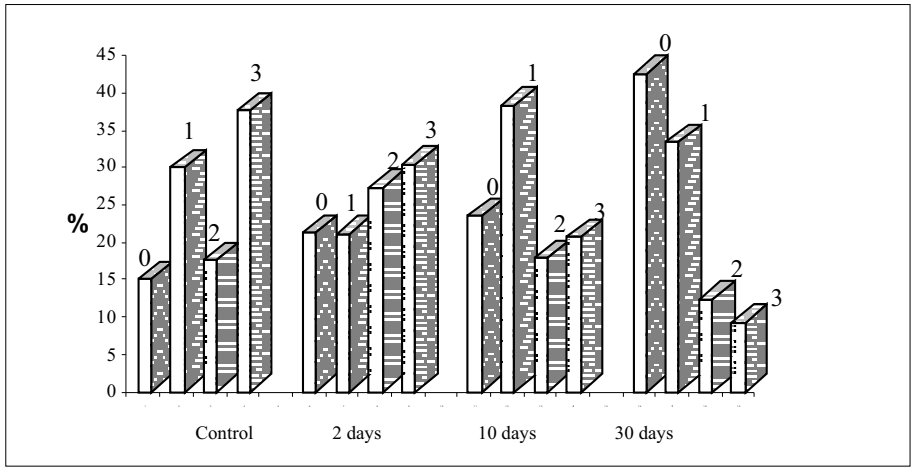

Fig. 2. Intensity of spongiosis at 3.2 DPCP site in regard to time of irradiation: 0 – no spongiosis; 1 – slight edema no intraepidermal vesicles; 2 – edema and single intraepidermal vesicles; 3 – severe edema and multiple intraepidermal vesicles.

The highest intensity of spongiosis (Fig. 2) was observed in sensitized and unirradiated volunteers from control group (34/40). The longer irradiation time, the lower intensity of spongiosis was observed. In a 2-day group no feature of spongiosis was observed in 26/33, in a 10-day group in 26/34 and in a 30-day group – in 19/33 cases. No spongiosis was found in skin samples taken from completely healthy skin (non-sensitized, non-irradiated). Histological pictures obtained from separate groups are shown as Figures 3–7.

We found a positive, statistically significant correlation between intensity of spongiosis and clinical score for CHS response at 3.2 DPCP site ($p<0.000001$). Moreover the same correlation between sum clinical score for CHS response (from all DPCP concentrations in all the volunteers) and 3.2 DPCP spongiosis intensity was observed ($p=0.00004$).

When both parameters (time of irradiation and spongiosis score at 3.2 DPCP site) were treated as continuous variables we revealed a negative correlation between these two parameters ($R=-0.28$; $p<0.001$).

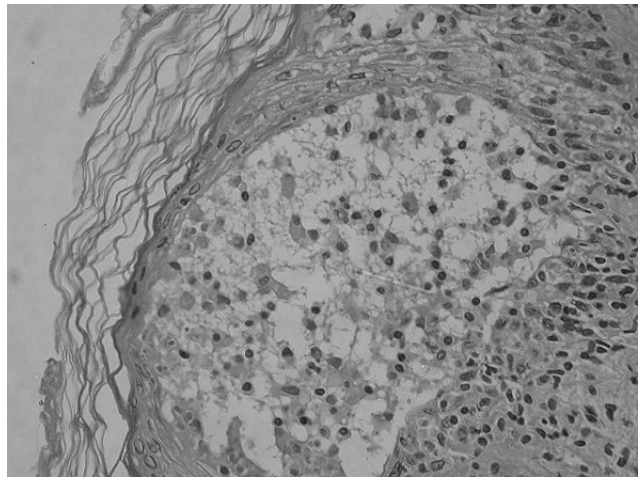

Fig. 4. Sensitized, non-irradiated group. HE. Magn. 400×.

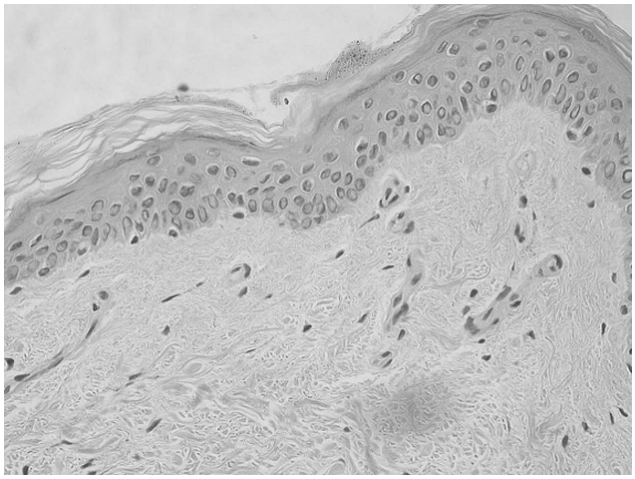

Fig. 3. Non-sensitized, non-irradiated skin. HE. Magn. 400×.

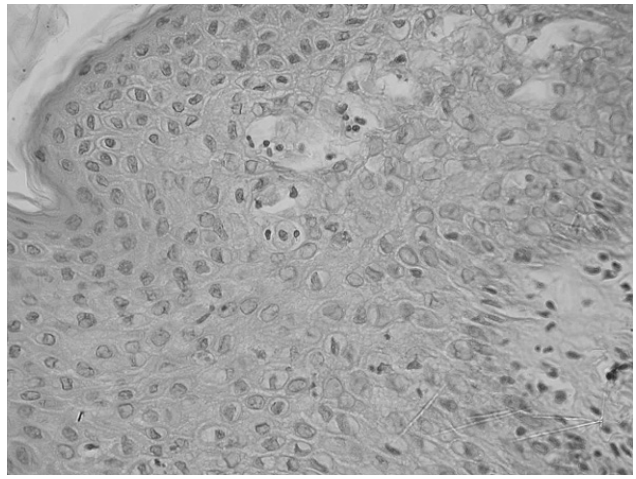

Fig. 5. 2-day irradiated, sensitized group. HE. Magn. 400x.

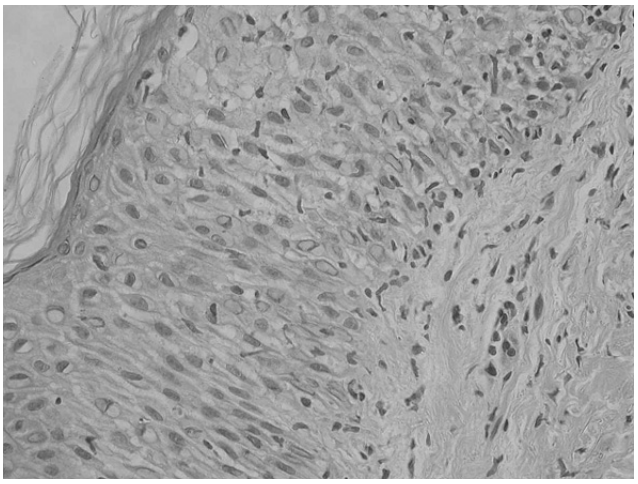

Fig. 6. 10-day irradiated, sensitized group. HE. Magn. 400×.

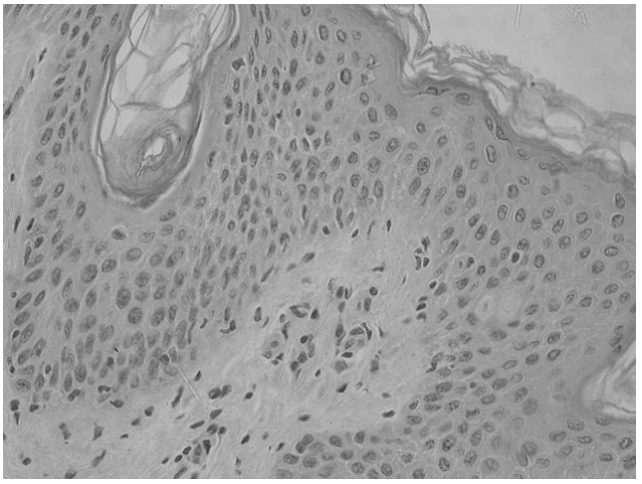

Fig. 7. 30-day irradiated, sensitized group. HE. Magn. 400×.

Discussion

In our study we focused on comparison and usefulness of two methods: visual scoring system and histological morphometry in the evaluation of CHS intensity after UVR. As our experiment was carried out in healthy human subjects, we were obligated to design the most accurate protocol, still acceptable by local ethics committee.

It should be stressed that a uniform method of CHS assessment response has not been established for human subjects, as yet. The standard protocol in mice for monitoring the intensity of CHS is to measure edema in the ear or footpad using a spring micrometer [11]. The most common method used by clinicians is a visual score reflecting the magnitude of the CHS response. However it is not objective and sensitive enough and it is possible that two different researchers may classify the same response in a little different way. Two research groups: i.e. Cooper et al. [2] and Skov et al. [13] employed skin-fold thickness for evaluation of CHS response, but such measurements are time-consuming and may vary with the operator, in addition to being problematic when edema and blistering are present.

Another method is a reflectance spectrometer which measures erythema, and skin pigmentation. Such reflectance devices have been used successfully in measuring the CHS response to nickel [3, 9]. However, erythema measurement, especially when severe CHS reaction occurs may be quite unreliable because the edema and blisters may influence the accuracy of readings. Kelly et al. [7] recommended the use of a high-frequency ultrasound scanner to determine the extent of CHS response. Unfortunately, because of economical reasons this equipment is not widely used in dermatological practice.

To verify clinical CHS assessment we performed histological analysis of thickness of the epidermis and the intensity of spongiosis based on its main features (i.e. the presence of edema and intraepidermal vesicles). The thickness of the epidermis correlated positively with the sum score of CHS response in all the groups ($p<0.05$). Significant correlation between intensity of spongiosis and clinical score for CHS response was also observed. We also found a negative correlation between time of irradiation and spongiosis score at 3.2 DPCP site ($p<0.001$).

Based on the obtained results we conclude that histological examination of biopsies taken from one of the series of elicitation sites is a reliable and sensitive method in the evaluation of CHS response after UVR. This method seems to be more precise than clinical assessment alone, however ethical reasons when scientific experiment is carried out in healthy subjects limit its use.

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