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SKIN PHOTOSENSITIZING AND LANGERHANS' CELL DEPLETING ACTIVITY OF TOPICAL (BATH) PUVA THERAPY: COMPARISON OF TRIMETHYLPSORALEN AND 8-METHOXYPSORALEN

Leena Koulu and Christer T. Jansén

Departments of Dermatology and Anatomy, University of Turku, SF-20520 Turku 52, Finland

Abstract. The skin photosensitizing and Langerhans' cell (LC) depleting effects of a single bath PUVA exposure were studied in 22 healthy young volunteers. The photosensitizing effect of bathing for 15 min in a 0.2 mg/l trioxsalen–water solution was about 30 times as great as a similar treatment in an equipotent methoxsalen solution. The skin erythema induced by methoxsalen bath PUVA peaked on day 2 and diminished therafter, whereas the trioxsalen reaction showed a broad plateau on days 2–5 after the irradiation. A reduction in LC density to about 30–40 % of the starting value was seen in both trioxsalen and methoxsalen bath PUVA treated skin sites on day 4 after irradiation, and low or diminishing LC counts prevailed until day 10–11. The amount of UVA needed to produce a similar degree of LC depletion was 15–30 times as great in the case of methoxsalen, compared with trioxsalen. A perceptible erythema reaction, however, was, not a prerequisite for a reduction in LC density.

Key words: Photochemotherapy; Photosensitization; Trioxsalen; Methoxsalen; Langerhans' cells

Psoralen photochemotherapy (PUVA) is a widely used treatment modality, effective in a variety of inflammatory and hyperproliferative skin disorders (10). The exact mode of action of PUVA therapy at the level of skin tissue is still debated and may vary in different skin conditions. Recently attention has been drawn to the marked Langerhans' cell (LC) depleting effects of PUVA (3, 7), which could possibly serve as an indicator for the therapeutic effectiveness of psoralen photochemotherapy.

While the most widely used PUVA regimen includes oral administration of the psoralen (systemic PUVA), topical application of the drug, by bathing in a dilute water solution of the psoralen (bath PUVA), has been shown to be a convenient and effective alternative (1, 4, 12, 16). For bath PUVA treatments trioxsalen has been used in the majority of centres, but its use is hampered by the very strong photosensitivity induced, which easily inflicts a phototoxic burn on the patient, either from the therapeutic UVA irradiation or from inadvertent solar exposure (1, 12, 16). Recently the less potently photsensitizing methoxsalen has been shown to be a useful alternative (5).

To compare more closely the performances of trioxsalen and methoxsalen in a bath PUVA procedure, we have studied the skin photosensitizing and Langerhans' cell depleting potencies of these two drugs in healthy volunteers.

MATERIAL AND METHODS

Photosensitivity testing

22 human volunteers (12 female, 10 male, 22–24 years of age) participated in the experiments. To sensitize the skin, one lower arm of each test subject was bathed for 15 min in a 0.2 mg/l water solution of trioxsalen (4,5',8-trimethylpsoralen; Star Ltd., Tampere, Finland) and the other arm in an equal strength of methoxsalen (8-methoxypsoralen; Star Ltd.). The psoralens were stored as stock solutions of 40 mg of trioxsalen or 80 mg of methoxsalen in 100 ml of ethanol, and the final bath solutions were prepared by diluting this stock solution in water. Immediately after bathing, the skin was wiped dry with a towel and 1 cm^2 squares on the volar aspects of the lower arms were exposed to graded series of UVA form a bank of Airam LUA fluorescent tubes emitting 6 mW/cm^2 of UVA radiation at the distance of the skin test site. On the trioxsalen bathed skin, doses ranging from 0.14 to 1.60 J/cm^2 and on the methoxsalen bathed skin doses from 1.6 to 18.2 J/cm^2 were used, in each case employing a geometrical progression of the doses with an increase factor of $\sqrt{2}$. The degree of skin erythema was recorded daily for 8 days using the following quantitation: no reaction =0, faint erythema with indefinite borders = 0.5+; moderate erythema = 1+; vivid erythema = 2+; erythema with edema = 3+.

To calculate the 1+ erythema time sequence (Fig. 1) the following approximation was used: when the largest dose given in a particular irradiation series showed less than 1+ response on a given day, the 1+ reaction was taken to be

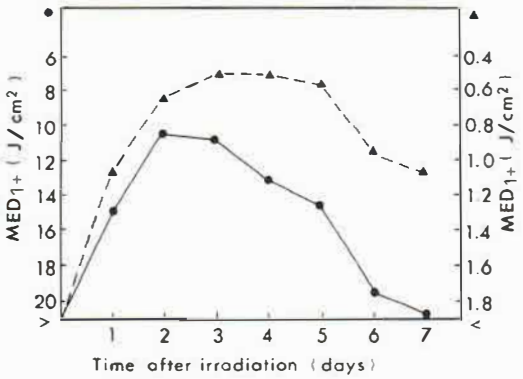

Fig. 1. Minimal 1+ erythema reaction causing UVA doses 1–7 days after bathing in a psoralen solution and application of a series of graded UVA irradiations. Trioxsalen-bathed skin = ▲, methoxsalen-bathed skin = ●. Medians of the minimal erythema doses (MED_{1+}) were calculated from readings on those 10 methoxsalen-bathed and 19 trioxsalen-bathed subjects in whom a 1+ reaction was recorded on one or more days of observation.

represented by the next (theoretical) exposure dose in the geometrical progression used, i.e. 2.26 J/cm² for trioxsalen and 25.6 J/cm² for methoxsalen. For comparison of the overall strength of the erythema reactions in different individuals (Fig. 2), the erythema scores of all positive sites of the irradiation series on day 3 were summed up to make an erythema sum score.

Langerhans' cell measurements

LC quantifications were made from punch biopsies taken from different subjects at different time points, ranging from 4 to 19 days post bath PUVA treatments. Sites which had reacted with a 1+ or 2+ erythema (on the day of the maximal erythema development) were primarily chosen for biopsying. In irradiation series where no 1+ reaction was achieved, the site of the greatest UVA dose was selected.

LC were stained by the ATPase method (6) using epidermal sheets separated from the biopsy samples by EDTA. The LC numbers were counted from 20 fields by projecting the stained epidermal specimen on a Reichert microscope projector screen at a magnification of ×200. For photography and morphological observations of the LC, a Leitz microscope was used at a magnification of ×400.

RESULTS

Skin photosensitivity

With the UVA doses used, 19 of the 22 test persons reacted with an erythemal reaction of 1+ or more in the trioxsalen series, while only 10 showed such a reaction in the methoxsalen series. Fig. 1 shows the evolution of the erythema over the 7-day period after the bath PUVA treatment. The skin erythema induced by methoxsalen bath PUVA peaked on day 2 and diminished therafter, whereas the trioxsalen reaction showed a broad plateau on days 2–5 after the irradiation.

The amount of UVA irradiation required to elicit a 1+ reaction differed considerably for the two psoralen drugs. When calculated for all the 22 test series for each drug, the median 1+ erythema (MED1+) eliciting UVA dose was 0.65 J/cm² for trioxsalen and 19.3 J/cm² for methoxsalen as registered on day 2; on day 3 the corresponding val-

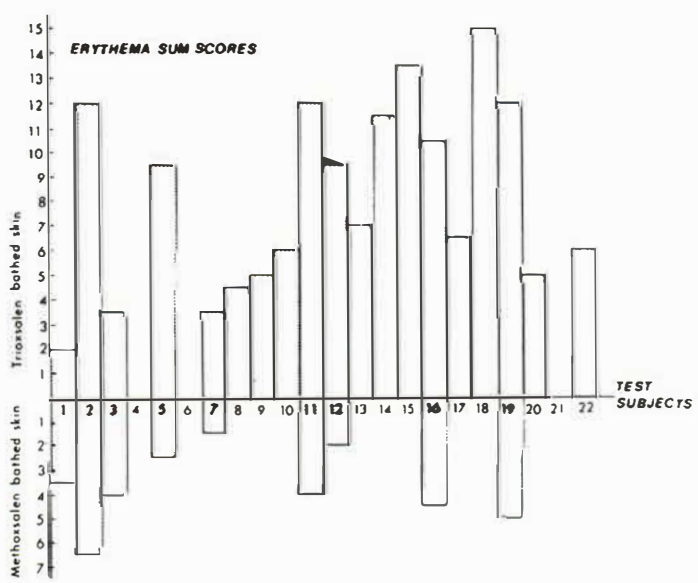

Fig. 2. Erythema sum scores on day 3 after a single bath PUVA treatment with trioxsalen or methoxsalen.

Table 1. *ATP-ase positive Langerhans' cell densities after a single bath PUVA treatment*

Time after irradiation (days)	Trioxsalen-bathed skin			Methoxsalen-bathed skin		
	No. of cases	Mean UVA dose at site of biopsy (J/cm^2)	LC count[a] ($cells/mm^2$)	No. of cases	Mean UVA dose at site of biopsy (J/cm^2)	LC count[a] ($cells/mm^2$)
Controls[b]	12	0	740±74	12	0	740±74
4	11	1.20±0.38	212±172	8	16.2±2.8	310±112
7–8	7	1.25±0.38	172±129	6	16.4±2.8	225±135
10–11	4	0.65±0.13	175±154	4	18.2±0.0	133±128
19	1	0.57	690	1	18.2	685

[a] Average of mean LC counts of the test subjects, ± SD.
[b] Counted from 12 age-matched control persons.

ues were 0.51 and 19.3 J/cm^2. Thus, the skin sensitizing capacity of the trioxsalen solution was about 30 times that of the equipotent (0.2 mg/l) methoxsalen solution. Great interindividual differences in skin sensitization were recorded, however, with both trioxsalen and methoxsalen, as shown by the erythema sum scores in Fig. 2. Furthermore, there was no significant correlation in the erythema reactions induced by the two psoralens (correlation coefficient 0.486, $p>0.1$).

Langerhans' cell depletion

After the single bath PUVA exposure a considerable reduction (to 30–40% of the starting value) in LC density was observed on day 4 at both trioxsalen- and methoxsalen-treated skin sites (Table 1). Thereafter a low or diminishing LC count prevailed until days 10–11, with a restoration to near the starting value by day 19. While the time sequence and depth of LC depletion caused by the two psoralens paralleled each other closely in the analysed skin specimens, the amount of UVA irradiation needed to produce the reaction was 15–30 times greater in the case of methoxsalen vis-à-vis trioxsalen (Table 1). The changes in LC morphology induced by the two regimens were very similar, the remaining Langerhans' cells being enlarged and displaying elongated dendritic processes (Fig. 3).

When LC depleting and photosensitizing effects of the treatments were compared, some interesting features emerged. Firstly, a clearcut reduction in LC counts was obtained even at skin sites not showing any perceptible erythema during the 8-day observation period (Fig. 4). Secondly, in all groups of erythemal responses (0, 1+, 2+, 3+) the LC depletion varied considerably in different subjects (Fig. 4).

DISCUSSION

Bath PUVA treatment, originally introduced in 1976 by Fischer and Alsins (1), is attracting increasing interest as an alternative to oral psoralen photochemotherapy, due to several practical advantages of the topical psoralen administration over that of oral medication. Because only minute quantities of psoralen are absorbed from a dilute water bath (2, 12, 17), problems of nausea, liver toxicity and eye sensitization are avoided and the patients need not wear UV protective goggles after the PUVA exposure.

Our knowledge of the pharmacokinetic characteristics of psoralen bath photochemotherapy are

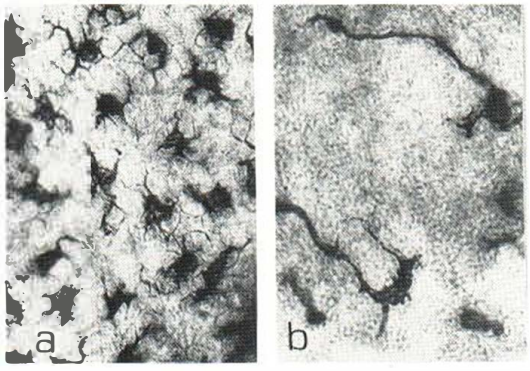

Fig. 3. Effect of a single bath PUVA treatment on epidermal ATP-ase positive Langerhans' cells. a) Non-exposed control skin, b) methoxsalen bath + 18.2 J/cm^2 of UVA. (*a*), (*b*), ×400.

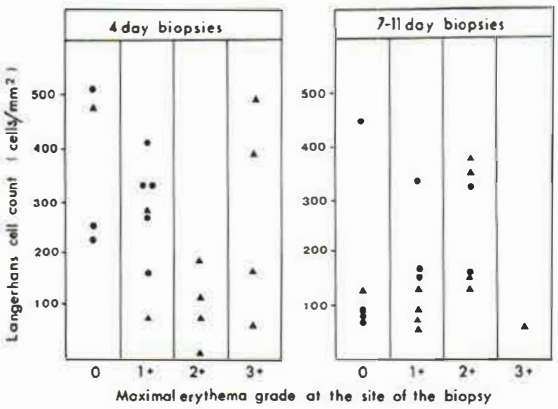

Fig. 4. Relation between the degree of erythema and LC depletion induced by a single bath PUVA exposure. Trioxsalen series = ▲, methoxsalen series = ●.

still insufficient, however, and comparative studies on various psoralen compounds have been dealt with in only one previous bath PUVA report (1). In the present study the peak development of the skin erythema was on days 2–3 post irradiation, which is in agreement with reports on oral methoxsalen (9) and studies on topical application of more concentrated psoralen solutions (1). In our series the phototoxic reaction from trioxsalen seemed to last longer than that from methoxsalen (Fig. 1). This might have some clinical implications in favour of methoxsalen, when considering repeated bath PUVA exposures within 1–3 days intervals.

As regards the relative photosensitizing potencies of the two drugs, trioxsalen has been advocated as a more potent topical sensitizer than methoxsalen in studies using topical painting of the substances (1, 8, 11), but comparative data from dilute water bath treatments have been lacking. Our finding that 15 min of bathing in a 0.2 mg/l water solution of trioxsalen was about 30 times more photosensitizing than a similar treatment with methoxsalen is noteworthy and has important practical implications. Fischer & Alsins compared the relative photsensitizing potencies of locally painted alcohol solutions of 5–500 mg/l of trioxsalen and 10–1000 mg/l of methoxsalen on human skin (1). In their study, trioxsalen sensitized the skin about five times more strongly than methoxsalen. The 6-fold difference in the sensitization ratio obtained in their study and in ours could be explained for example by the differing absorption properties of the two psoralens from alcoholic solution painting vs. water bathing.

It is well known that oral photochemotherapy produces, in different persons, widely varying blood concentrations as well as differing skin photosensitivity levels (13, 14). Our data show that the same kind of variance is found in skin photosensitivity levels obtained after bathing in trioxsalen or methoxsalen water solutions (Fig. 2). This is in accordance with the data of Väätäinen & Taskinen (17) who showed up to a 20-fold variation in the skin drug content between different persons after bathing in a 0.3 mg/l water solution of trioxsalen. In addition, our data demonstrated a surprising lack of correlation between the levels of trioxsalen and methoxsalen skin absorption as judged from the erythemal responses.

Our data on Langerhans' cells corroborate earlier findings of a reduction in human epidermal LC counts during PUVA therapy whether by oral medication (3, 7) or with a bath regimen (15). The single bath PUVA exposures used in our study caused an approx. 70% reduction in the LC counts, apparent on days 4–10 post irradiation, with restoration to near control values by day 19 (Table I). These results are similar to those obtained by us in studies when using oral methoxsalen (7). It is not known whether psoralen-induced erythema and LC depletion are interrelated or independent photobiological phenomena. Our study shows that a clinically perceptible erythema was not required for the PUVA exposure in order to produce a considerable reduction in the LC count (Fig. 4). In most such cases, however, a faint pigmentation developed as a macroscopically evident skin reaction (data not shown).

A most important, still unresolved matter is the question of whether the antipsoriatic effects of PUVA therapy are dependent on either the phototoxic or the LC depleting properties of psoralen photochemotherapy. Although trioxsalen-bathed patients often develop erythema during the PUVA course (1, 16, 12), good therapeutic results have been obtained with methoxsalen baths when using doses considerably smaller than the phototoxicity levels found in the present study (5). Fischer & Alsins also reported total clearing of psoriasis with methoxsalen bath PUVA using doses only about twice as high as required for clearing psoriasis with trioxsalen bath PUVA (1). Clearly, more studies are warranted to investigate the relationship between the therapeutic effectiveness and photosensitizing and LC depleting activities of different photochemotherapy regimens.

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Leena Koulu, M.D.
Department of Dermatology
University of Turku
SF-20520 Turku 52, Finland