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TREATMENT OF PSORIASIS WITH ORAL PSORALENS AND LONGWAVE ULTRAVIOLET LIGHT

Therapeutic Results and Cytogenetic Hazards

Gunnar Swanbeck, Monika Thyresson-Hök, Anders Bredberg and Bo Lambert

From the Departments of Dermatology and Clinical Genetics, Karolinska sjukhuset, Stockholm, Sweden

Abstract. The purpose of the present investigation was to study the usefulness of oral treatment of psoriasis with psoralens and longwave ultraviolet light and the possible cytogenetic hazards of this therapy. 8-methoxypsoralen (8-MOP) in doses between 15 and 60 mg orally followed 2 hours later by UVA irradiation of one side of the body gave a healing of the irradiated side in 24 of 40 cases and an improvment in another 11 cases while only one case healed on the side of body that was not irradiated. The most common undesired side effect was pruritus on the irradiated side of the body. The cytogenetic study showed that 8-MOP and UVA treatment of lymphocytes in vitro gives rise to chromosomal aberrations. In a combined in vivo – in vitro study where the lymphocytes had been isolated from a patient 2 hours after intake of 60–80 mg 8-MOP and then irradiated with therapeutic UVA doses, a significant increase in chromosomal aberrations was found. When chromosome analyses were made on the patients whilst the 8-MOP treatment was temporarily withdrawn and when the lymphocytes were not irradiated in vitro, no increased frequency of chromosomal aberrations was found on comparison with a group of psoriatic patients receiving dithranol therapy.

Key words: Psoralens; Ultraviolet light; Psoriasis; Chromosomal aberrations

The positive results of local treatment with psoralens and longwave ultraviolet light (UVA) (7, 21, 22, 25, 26) made us interested in studying whether also this antipsoriatic agent might be a potent inducer of mitochondrial mutations in yeast in the same way as are dithranol (9, 27), coal tar extracts (Swanbeck, G. & Zetterberg, G.: Unpublished results), and methotrexate (19). Upon finding that this was the case (20), we commenced experimental treatment of psoriatic patients with oral psoralens and UVA. To administer a relatively inert substance systemically and then activate it in the skin by harmless irradiation is a very interesting principle, which has been favorably applied earlier in the treatment of vitiligo with just oral psoralens plus UVA irradiation (6, 11).

During the preparation of this paper a report of successful treatment of psoriasis with oral methoxalen and UVA was published by Parrish et al. (16), but we have also found two earlier reports on this type of treatment (15, 23). Although it seems possible that one day patients will be able to self-administer this treatment in their homes, there are practical problems with regard to suitable light sources. Another problem is the safety of the treatment regarding the effect on the liver and chromosomes.

It seems highly probable that the treatment acts by a direct effect on DNA. Psoralens react both in vitro and in vivo with pyrimidine bases of DNA under irradiation, producing monofunctional and bifunctional additions or interstrand cross-links in native DNA (5, 12, 13). An increased frequency of chromosome aberrations in human lymphocytes (18) and the induction of DNA repair synthesis in human fibroblasts (2) have been reported after 8-methoxypsoralen (8-MOP) plus UVA treatment in vitro. Against this background, it is important that studies should be made on genetic effects in connection with psoralen treatment of psoriasis patients, especially when one considers that this treatment does not provide a definite cure but only a temporary removal of the skin lesions. Thus a series of treatments may be repeated several times. It is also probable that a very large number of patients will be able to use this apparently convenient treatment for a considerable length of time. In the present paper we present our clinical experience of treating psoriasis with psoralens and UVA and the chromosome studies we have made so far.

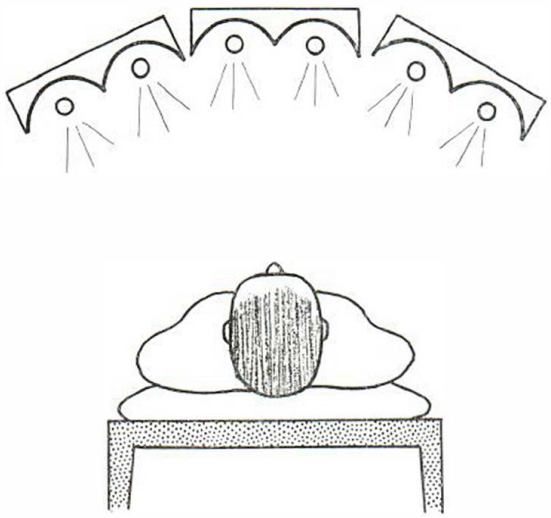

Fig. 1. Schematic picture of the irradiation treatment. Each circle represents a 40 W, 120 cm long black light tube.

MATERIAL AND METHODS

Clinical Study

Patients

Patients who have had psoriasis for several years and where other therapeutic measures such as topical steroids and anthralin had not given satisfactory results were selected for this investigation. One patient had earlier only used emollient cremes and sun lamp irradiation. Several patients had earlier been treated with hydroxyurea and/or methotrexate. We did not regard photo sensitivity as an absolute contra-indication for treatment with psoralens and UVA and included in the investigation 2 patients who reported that their psoriasis was usually worse during the summer period. Only patients who were healthy apart from having psoriasis were included.

Medication with psoralens

Initially, trimethylpsoralen (TMP), (Trisoralen®, Paul Elder Co, Bryan, Ohio, USA) was given to 3 patients, 40 mg daily. Subsequently we only used 8-methoxypsoralen (MOP) (Neo meladinine, The Memphis Chemical Co, Cairo, Egypt), 15 mg tablets. The maximum doses given were 30 mg to patients weighing 35–55 kg, 45 mg to patients weighing 55–75 kg and 60 mg to patients weighing more than 75 kg. When an irritating erythema, pruritus or nausea occurred the dose was diminished by one tablet.

Radiation treatment

The irradiation was done with six 40 W fluorescent black-light tubes (Philips TL 40 W/08) with suitable reflectors. The irradiation has a spectral maximum at 360 nm and practically no radiation below 320 nm. The lamps were arranged as shown in Fig. 1, with a distance of 40 cm between lamps and patient. Intensity at body surface has been estimated to be approximately 3 mW/cm². The irradiation time was generally 30 minutes, giving a total dose of about 5.4 J/cm².

Treatment schedule

Between 2 and 2.5 hours after ingestion of the tablets, the patients were irradiated on either the ventral or dorsal side of the body, which ever side had the more severe or extensive psoriatic lesions. The other side was not irradiated before the first side was completely healed but served as a control with respect to placebo effect or coincidental spontaneous healing. The patients were treated 5 days weekly. The maintenance treatment on the treated side after healing was given once a week or less frequently to begin with. If new lesions appeared, the treatment was given more frequently. The control side was treated at the same time as the maintenance treatment of the first-treated side proceeded. The so-called control side served as a control only as long as the initial treatment of other side proceeded, but not during the maintenance treatment. The results of the subsequent treatment of the control side are not reported here. In this way we have tried to obtain information on how often maintenance therapy was necessary.

Evaluation of clinical effect

We have regarded the treated side as healed when no lesion could be palpated or no erythema remained. We have regarded the treated side as improved if all lesions had definitely become less elevated and less scaling. If the treated side of a patient did not fulfil the criteria for either healing or improvement, the patient was classified 'unchanged, or 'worse'. The scalp has not been included in this investigation as it is difficult to irradiate.

Laboratory controls

All patients were checked weekly with regard to hemoglobin concentration, white blood cell count, differential count, thrombocyte count, transaminases, bilirubin, and alkaline phosphatase determination in serum.

Cytogenetic Study

In vitro experiment

Peripheral leukocytes were obtained by centrifugation from freshly collected, heparinized venous blood from one healthy subject. The cells were washed in phosphate-buffered saline (PBS, Flow Laboratories) and resuspended in Parker 199 (Flow Laboratories) supplemented with 25% foetal calf serum, 125 μg/ml streptomycin and 125 IE/ml benzyl penicillin. Subsequent work was done in complete darkness when possible, or otherwise in red darkroom illumination. Each of 5 identical cultures in 30 ml Falcon plastic flasks contained 8-MOP in a final concentration of 26.7 μg/ml (A–C of Table IV) or 0.1 μg/ml (D and E of Table IV). The drug was allowed to penetrate into the cells for 15–30 min, and the cultures were then irradiated with longwave UV-light (365 nm, 5 mW/cm²) through the Falcon flasks, which were found to transmit 100% of the light. Exposure was between 30 sec and 14 min, to yield a final dose range between 0.15 and 4.2 J/cm² in the various cultures. After irradiation the cells were transferred to centrifuge tubes and washed 3 times, 5 min each, in PBS to remove unbound 8-MOP. Conventional lymphocyte cultures with phytohemagglutinin (0.02 ml of PHA, Wellcome, per ml culture medium) were set up and run for 50 or 74 hours. Colchicine (0.2 μg/ml) was added 2 hours before harvesting. After

hypotonic treatment (0.075 M KCl) and fixation (methanol/acetic acid, 3:1) conventional preparations were made and air-dried. The slides were stained in Giemsa (5% in 0.01 M phosphate buffer, pH 6.8) and scored for well spread metaphases.

In vivo studies

Venous blood was obtained and collected in light-sheltered, heparinized test tubes from 8 of the patients receiving the 8-MOP+UVA therapy, 5 patients subjected to dithranol treatment and 6 healthy non-psoriatic controls. Conventional lymphocyte cultures with PHA were set up as described above and run in the dark for 72 hours. Colchicine (0.1 μg/ml) was added 2 hours before harvesting. Fixation and preparation of cells were carried out as in the in vitro experiment. The cultures were coded and subsequent chromosome analyses were made blind.

In the combined in vivo – in vitro studies, 40 ml of venous blood was drawn from each of 4 patients 2 hours after ingestion of 60–80 mg of 8-MOP. The plasma suspension of leukocytes was isolated after centrifugation of the blood, and 5 ml aliquots were placed in 3 Falcon flasks. Two of the flasks were exposed to UVA-light for 3.5 and 14 min, respectively. The UVA-doses were chosen so as to facilitate comparison with the doses given in the in vitro experiments and with the estimated therapeutic dose (see Discussion). The third flask was not irradiated but served as dark control. In a parallel experiment the plasma suspension of leukocytes from a subject not receiving psoralen therapy was irradiated to investigate the effect of UVA-light alone on the frequency of chromosome aberrations. After irradiation the cells were cultured for 72 hours as described above, and chromosome preparations were made as in the other studies.

Chromosome analysis and definitions of aberrations

Between 7 and 14 slides, depending on the number and quality of mitotic cells, were prepared from each culture. With exception of the in vitro study, all slides were coded and subsequent analysis was done blind. Suitable metaphases were selected at random at low magnification, and the detailed analysis was then carried out at higher magnification.

The following definitions were adopted: a gap is an achromatic lesion in an otherwise intact chromatid without displacement of the distal segment. A break is stated only when the distal segment is dislocated from the chromosome axis. Isochromatid gaps and breaks are lesions which affect both sister chromatids at the same level. A metaphase containing an acentric fragment with or without a chromosome deletion was scored as one break. Chromatid exchanges, translocations, inversions, dicentrics and ring chromosomes were each scored as one aberration, although they should be regarded as the result of several simultaneous breaks in the same cell. Because of the greater difficulties involved in the evaluation of gaps, only breaks and structural rearrangements were considered as true aberrations in the tabulation of the results. In Tables IV, V and VII, *g′* stands for chromatid gap, *g″* for isochromatid gap, *b′* for chromatid break and/or single fragment, *b″* for isochromatid break and/or double fragment. "Others" stands for chromatid exchanges, translocations, inversions, dicentrics and ring chromosomes.

RESULTS

Clinical Study

Three patients were treated with TMP, 40 mg, 5 days weekly, for 3, 4, and 6 weeks respectively. No erythema or effect on the psoriasis was noted. For 2 of these patients the skin lesions healed in 3 weeks upon switching to 30–45 mg MOP daily 5 days a week. The third patient healed after 5 weeks on the same treatment.

Subsequently only 8-MOP was used in 40 patients and the results are given in Tables I and II with regard to the therapeutic effect. The data given in Table I refers to the UVA-irradiated side of the patient. In only one case did the non-irradiated side also heal—4 weeks after treatment of the opposite side had started. Thus 23 of 40 patients healed on one side only. None of these patients healed on the unirradiated side and 23 healed on the irradiated side. The observed relative frequency of the latter event is thus 23/40 = 0.575 with a 95% confidence interval ranging from 0.42–0.73.

In Table I the numbers of patients who were healed after 3, 5, 7 and 9 weeks treatment are given. The number of patients who, up till the end of the period of the clinical trial, were not healed but improved is also given. The patients in this group had been treated for 5 weeks or more which means that some of them might have been healed when treated for totally 7 or 9 weeks. Five patients did not respond positively to the therapy. In this group are included those patients who had to break off their treatment because of some undesirable side effect. Of these 5 patients, a 28-year-old woman interrupted the treatment because of nausea. Two patients whose psoriasis worsened during the summer months also deteriorated during this treatment and therefore dropped out. One 42-year-old woman earlier taking

Table I. *Therapeutic results of the 8-MOP and UVA treatment*

Healed[a]				Improved[b]	Not improved or worse	Total
3w	5w	7w	9w			
5	15	20	24	11	5	40

[a] Number of patients healed within the time period indicated (cumulative figures).
[b] Improved but not completely healed after more than 5 weeks' treatment.

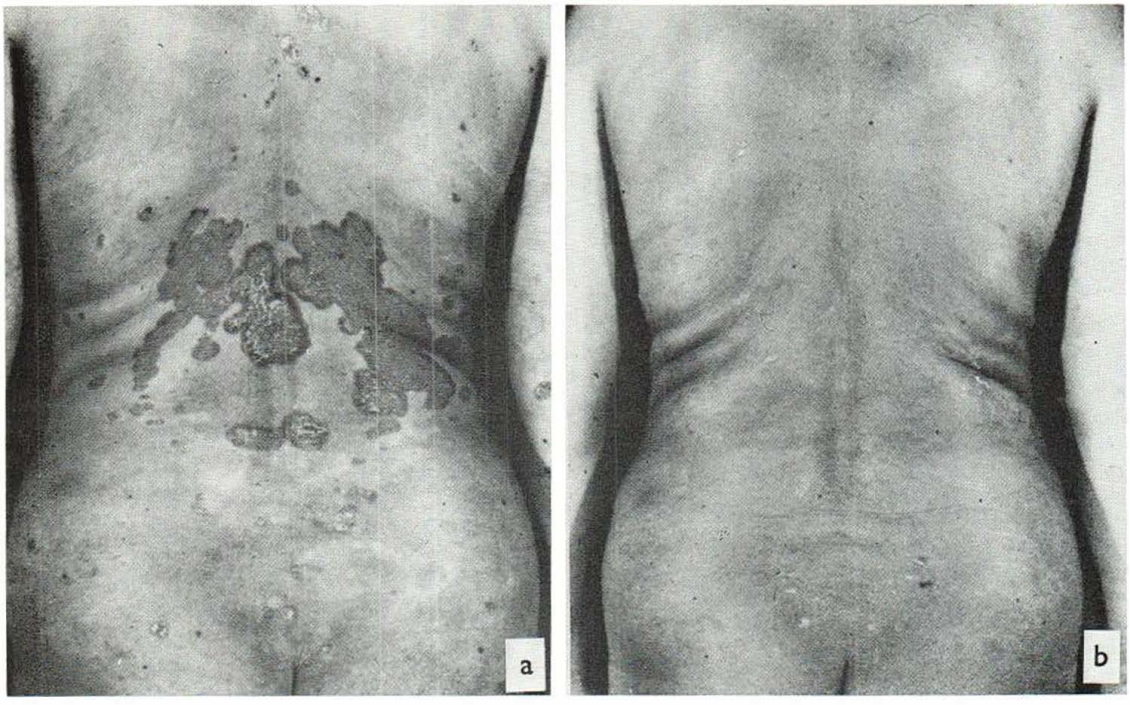

Fig. 2. The appearance of one of the patients before (a) and after (b) treatment with 8-MOP and UVA.

hydroxyurea, which was suspended when the 8-MOP treatment started, became worse; the 8-MOP treatment was discontinued and hydroxyurea reinstituted. The fifth patient was a 71-year-old woman who failed to respond in any way to 60 mg 8-MOP plus UVA, showing neither erythema nor healing of her lesions.

Table II indicates how often treatment had to be given to the 17 patients who had been on maintenance treatment for more than 3 months. Nearly half of this group could not be kept free of new lesions with only one treatment per week.

All healed patients and most of the improved patients were very enthusiastic about this type of treatment. The frequency of undesirable side effects is given in Table III. An intense pruritus was experienced by 7 patients who in the beginning of the treatment got an intense erythema on uninvolved skin. In 6 patients the serum transaminase activity was transiently slightly increased. Three of these patients admitted that they were taking alcohol frequently. In 2 patients without lesions on the buttocks, intense erythema appeared at this site, as also did a subsequent Koebner reaction which, however, healed after continued treatment with a lower 8-MOP dose.

Table II. *Maintenance treatment necessary to keep new lesions from appearing*[a]

1/week	4–5/week
9	8

[a] Estimated during at least 3 months' maintenance treatment.

Chromosome Analyses

The frequencies of chromosome aberrations after various combinations of 8-MOP and UVA exposure was studied in vitro in order to establish a preliminary

Table III. *Undesirable side-effects*

Koebner reaction after intensive erythema	2
Pruritus	7
Nausea	1
Transient increase in serum transaminase	6

Table IV. *Chromosome aberrations in lymphocytes treated with 8-MOP + UVA* in vitro

Code and culture time (hours)	Treatment		No. of cells analysed	No. of gaps[a]		No. of different types of aberrations[a]			Total no. of aberrations	Aberrant cells (%)
	8-MOP (μg/ml)	UV dose (J/cm²)		g′	g″	b′	b″	Others		
A50	26.7	none	50	7		2			2	2
B50	26.7	0.15	50	9	3	2			2	4
C50	26.7	0.6	—[b]						—	—
D50	0.1	1.05	50	5	1	1			1	2
E50	0.1	4.2	30	2		4			4	10
A74	26.7	none	100	10		1			1	1
B74	26.7	0.15	100	15	3	18	7	6	31	25
C74	26.7	0.6	62	43	3	52	16	10	78	66
D74	0.1	1.05	100	12	5	3			3	3
E74	0.1	4.2	100	17	4	14	5	3	22	19

[a] *g′* stands for chromatid gap, *g″* for isochromatid gap, *b′* for chromatid break and/or single fragment, *b″* for isochromatid break and/or double fragment. 'Others' stands for chromatid exchanges, translocations, inversions, dicentrics and ring chromosomes. Note that gaps are not considered as aberrations. For further details see Material and methods.
[b] No mitosis found. See Results.

dose–response relationship. The results are shown in Table IV and Fig. 3. Only a few 8-MOP + UVA treated cells were in mitosis after 50 hours of culture time. In those cultures where analysis could be carried out (B50, D50, E50) there was no increase in the frequency of chromosome aberrations, compared with the control (A50). The low number of mitoses is probably due to a delaying effect on the DNA replication due to DNA damage caused by the 8-MOP + UVA treatment (3).

Cells not so severely damaged may have escaped the mitotic delay, and would thus dominate among mitotic cells after 50 hours, while more severely damaged cells would be delayed and not show up. This may explain the relatively low number of chromosome aberrations after 50 hours of culture time compared with the aberration frequency after the same treatment in the 74 hour cultures. The number of aberrant cells in cultures run for 74 hours was considerably increased in all treated cultures, compared with the control (A74). There was an evident effect of increasing both 8-MOP concentration and UVA dose on the frequency of chromatid breaks (Fig 3, Table IV). However, aberrations of the isochromatid or chromosome type were also significantly increased. Between 3 and 16% of the cells in cultures B74, C74, and E74 demonstrated characteristic chromatid exchange configurations.

In the combined in vivo – in vitro experiment, peripheral blood was obtained from 4 patients 2 hours after they had each taken 60–80 mg of 8-MOP and from one healthy control who did not receive the drug. Irradiation was carried out in vitro and the cells were cultured for 74 hours. The results (Table V) indicate that the therapeutic in vivo concentration of 8-MOP in peripheral lymphocytes is high enough to produce an increased frequency of chromosome aberrations after exposure to UVA-doses of therapeutic magnitude. However, one patient did not respond with increased aberration

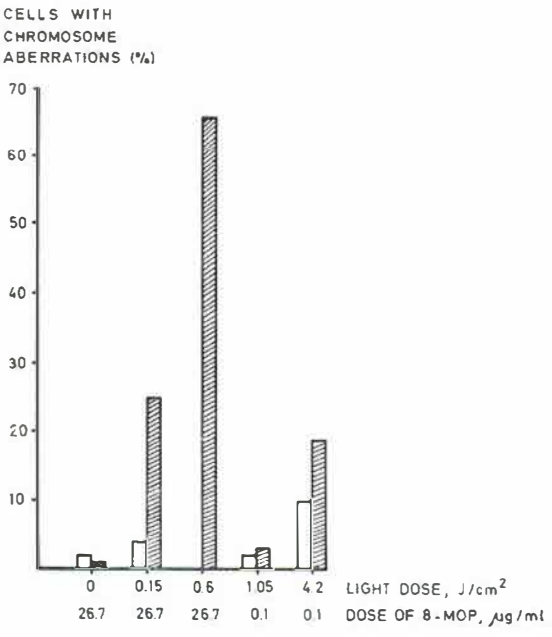

Fig. 3. Frequency of cells with chromosome aberrations in the in vitro experiment. ▭, 50 hours of culture time; ▨, 74 hours.

Table V. *Chromosome aberrations in the combined in vivo – in vitro study*

Subjects[a]	UV dose (J/cm²)	No. of gaps		No. of different types of aberrations			Aberrations per 100 cells	Statistical analysis[b]	
		g′	g″	b′	b″	Others		χ^2	P
P9	0	4			1		1		
P9	1.05	6	4		8		8	49.0	<0.025
P9	4.2		1		1	1	2		
P10	0	3			1		1		
P10	1.05	1	1				0		
P10	4.2	1		1			1		
P11	0		1		4		4		
P11	1.05	1	4		12		12	16.0	<0.05
P11	4.2	1	4		9		9	6.3	<0.2
P12	0		2	1	2	2	5		
P12	1.05		1		4		4		
P12	4.2	3	6		12	1	13	12.8	<0.05
C7	0				5		5		
C7	4.2	1			4		4		

[a] P = psoriasis patient under 8-MOP + UVA treatment, P9, P10 and P11 had received 60 mg of 8-MOP, and P12 80 mg. C = healthy control who had received no 8-MOP. 100 cells were analysed from each subject-UV dose.
[b] Expected value is that of the unirradiated sample of the same patient (one degree of freedom).

frequency at any of the UVA-doses, and in the other cases there did not seem to be any correlation between the UVA-doses given and the frequency of aberrations. The UVA-exposed lymphocytes from the control (C7) did not show an increased aberration frequency.

In the in vivo studies two groups of psoriasis patients were investigated. One group comprised 5 patients on dithranol therapy, and the other group comprised 8 patients receiving 8-MOP + UVA treatment. Several patients in both groups had previously received other types of therapy (see

Table VI. *Relevant data from the medical history of the patients included in the cytogenetic study (Tables V and VII)*

Case	Sex	Age	Age at onset of psoriasis	Earlier psoriasis therapy				8-MOP treatment	
				Arsenic	Methotrexate	Dithranol	Tar	Daily dose (mg)	Treatment time (weeks)
D1	♂	55	33	–	–	+	–	–	–
D2	♀	60	15	+	–	+	+	–	–
D3	♂	39	22	–	–	+	+	–	–
D4	♂	71	63	–	–	+	+	–	–
D5	♀	89	81	–	–	+	–	–	–
P1	♀	31	15	+	+	+	–	15–30	8
P2	♀	24	13	+	–	+	–	45	17
P3	♂	55	16	+	–	+	+	30	8
P4	♂	55	20	+	–	+	+	30	2
P5	♂	20	19	–	–	+	–	30	1
P6	♂	54	41	–	+	+	–	30	1
P7	♂	55	42	–	–	+	+	45–60	10
P8	♀	36	29	–	–	–	–	30	2
P9	♂	38	25	–	–	+	–	30	4
P10	♂	35	29	–	–	+	–	45	15
P11	♀	24	4	–	–	–	–	30	1
P12	♂	55	16	+	–	+	+	30	17

Patients P3 and P12 are the same individual, who was represented in both the *in vivo* and the combined in vivo – in vitro experiments. Patients P2, P3 and P8 had been treated with 40 mg of TMP for some weeks before the 8-MOP treatment.

Table VII. *Chromosome aberrations in controls and psoriasis patients*

Subjects[a]	No. of gaps		No. of different types of aberrations			Aberrations per 100 cells	Statistical analysis[b]	
	g'	*g''*	*b'*	*b''*	Others		χ^2	*P*
C1	6	2	5	2	1	8	5.1	<0.2
C2	3	2	2	1	1	4		
C3		1				0		
C4		5		2		2		
C5		3		5	1	6		
C6			1	1		2		
Mean						3.7		
D1	11	5	8	12	2	22	91.6	<0.01
D2	2	10		3	2	5		
D3	2	2	2	2		4		
D4	9	2	3	3	6	12	18.9	<0.05
D5	8	3	1	1		2		
Mean						9.0		
P1	2	7	1	4		5		
P2	1	1	1	1		2		
P3		4		6		6		
P4	5	2	2	3	1	6		
P5	8	1	4	2		6		
P6	8	2	3	4		7		
P7	3		2	6		8		
P8	10	3	9	8		17	48.4	<0.025
Mean						7.1		

[a] C = healthy controls, D = psoriasis patients under dithranol treatment, P = psoriasis patients under 8-MOP + UVA treatment. 100 cells were analysed from each subject.
[b] Expected values is the average of the control group (one degree of freedom).

Table VI). Blood specimens from the dithranol patients were obtained just before their daily treatment, and from the psoralen patients at least 24 hours after the previous 8-MOP + UVA treatment. Patients of both groups had undergone therapy for varying lengths of time before the chromosome analyses were undertaken (Table VI).

The control group comprised 6 apparently healthy subjects among the clinical and laboratory staff. Conventional PHA-stimulated lymphocyte cultures were set up, and the mitotic cells were analysed for chromosome aberrations after 74 hours of culture. The results are shown in Table VII. Four control subjects demonstrated fewer than 5% aberrations in 100 cells, which is in agreement with other series of control analyses in this laboratory (8) and with that of other studies (4). A higher aberration frequency than expected was recorded in 2 control cases (C1, C5). There were no incidents of viral disease, drugs, or previous X-ray in the history of these cases which might have explained the high aberration frequency, and reinvestigation has been initiated.

In the group of dithranol-treated psoriasis patients, 3 subjects demonstrated normal values, while in 2 cases (D1, D4) a much higher number of aberrations was found. The 8-MOP + UVA treated patients likewise demonstrated heterogeneity. One case (P2) had a normal aberration frequency, for 6 cases aberration frequencies slightly above normal values were recorded, and in one additional case (P8) the frequency of aberrations was increased. Thus within all groups there was a considerable heterogeneity with regard to the frequency of chromosomal aberrations.

DISCUSSION

Extensive psoriatic lesions are a physical and a great social handicap to the patients. The treatment of psoriasis is usually either messy or unpleasant, as in the case of coal tar and dithranol treatment, or associated with undesired side-effects as in the case

of local treatment with fluorinated steroids or systemic use of cytostatic agents.

Treatment with oral psoralens and UVA radiation is a systemic therapy with local effect. With suitable UVA light sources this is an agreeable and rather pleasant type of therapy that was very much appreciated by the patients. The UVA-light source used by us is commercially available and cheap. It requires up to 30 minutes' exposure time on each side, which limits its usefulness. A stronger light source has been used by Parrish et al. (16).

Although our experience is limited to 3 treated cases, we feel that systemic treatment of psoriasis with TMP in combination with UVA irradiation is of no value. When evaluating the efficacy of the treatment one has to take into account the great variability in therapy resistance of the lesions in different patients. In the present study we have deliberately chosen patients with rather severe psoriasis and have for the first 5 weeks of treatment irradiated only one side of the patient. The other side has functioned as the control side. In only one case did the untreated control side heal, whereas the treated side healed completely in 24 of the 40 patients, which is highly significant, statistically. In 34 of the 40 patients the treated side was better than the control side. It is thus clearly shown that this treatment has an antipsoriatic effect. No comparison with other types of treatment has been made in this study. Our impression is that this type of treatment is convenient and effective. The optimal interval between the treatments needs to be investigated further. Five treatments per week is efficacious but three treatments might give a better ratio between effect and side effects.

How often the patient has to be treated after healing in order to prevent new lesions from appearing apparently varies very much from patient to patient. Some patients definitely had to be treated more than once a week to avoid new lesions, while others only had to be treated once a week to avoid relapse during our rather short observation period. Many patients with less severe psoriasis may remain healed for a long time without maintenance treatment. In any case, the method seems to be technically suitable for maintenance treatment.

A positive side-effect is the pigmentation which on the uninvolved skin appears before the lesions heal. The hypopigmentation seen when the lesions disappear soon becomes pigmented and little difference in pigmentation can be detected between earlier lesion and uninvolved skin. A negative side-effect is the frequently itching erythema which appeared a few days after the first treatment. This may be avoided by starting with a lower dose of light or 8-MOP or both and then increasing slowly. In 2 patients a Koebner reaction was provoked, which healed during subsequent treatment.

Among the 6 cases with pathological serum transaminase values, 3 had temporarily taken alcohol during the treatment period. In the other 3 cases we cannot exclude that alcohol had been used during the treatment period. Whether the slightly pathological liver tests were due to alcohol alone, or alcohol in combination with 8-MOP, we do not know. However, until we know more about this problem, one should advise the patients not to use alcohol when on 8-MOP treatment as it may increase the risk of liver damage.

The most serious risk is probably to the skin, where we have the combination of 8-MOP and light which might cause damage to DNA (12, 13). The RD-mutation inducing ability of 8-MOP and UVA-light in yeast reported earlier is probably not alarming. We know that most other antipsoriatic agents have such an effect without being particularly harmful (9, 19, 20, 27). This type of mutation is due to an effect on mitochondrial DNA (1).

The main conclusion of the present in vitro and combined in vivo – in vitro studies is that the treatment of lymphocytes with 8-MOP + UVA light in therapeutic doses gives rise to a significantly increased frequency of chromosome aberrations. The type of chromosome aberration which may appear after DNA damage depends on several factors, viz. the type of DNA lesion and the cellular phase in which the damage is introduced, the number of replications which have passed until the cell is analysed, and the type of DNA repair process which is induced by the damage. In the present in vitro experiments, DNA damage was introduced in G0 phase, and all mitotic cells after 74 hours of culture time should be in their second round of division. The different types of chromosome aberrations which arise are therefore probably caused mainly by the combined effects of various types of DNA damage and cellular repair processes operating in pre- and post-mitotic phases. Thus the high frequency of chromatid aberrations and chromatid exchanges observed after 74 hours of culture time, could reflect persistent single strand lesions or single strand gaps

arising during post-replication repair of interstrand cross-links (10).

When 8-MOP is added to isolated human lymphocytes in vitro and the cells subsequently are irradiated with UVA, chromosomal aberrations appear in numbers that seem to be dose dependent both with regard to the 8-MOP dose and the light dose. If the lymphocytes are isolated from the blood of patients who have taken 60–80 mg 8-MOP orally 2 hours earlier and the lymphocytes then are irradiated in vitro with 1.05–4.2 J/cm² of UVA, a significant increase in chromosomal aberrations is obtained in three out of four cases. As this experiment is done under conditions similar to the in vitro experiment with regard to irradiation, we may infer that the content of 8-MOP in the serum of a patient 2 hours after taking 60–80 mg of 8-MOP is somewhat less than 0.1 μg/ml. This concentration in vitro gives about the same number of chromosomal aberrations as is obtained for the lymphocytes that have been treated in vivo with 8-MOP and subsequently in vitro irradiated. However, there was great variation in the frequency of chromosome aberrations between individuals, which may relate to differences in metabolism of 8-MOP and sensitivity to UVA-light. The combined in vivo – in vitro approach could possibly be useful for estimating the optimal, individual combination of 8-MOP and UVA dose for clinical use.

The light dose used in this combined in vivo – in vitro experiment was of the same order as the skin is receiving in the treatment of the patients. However, when the patients are treated there is some absorption of light in the horny layer and the spinous layer of epidermis, which probably cannot give any harmful genetic effects. It must also be pointed out that the in vitro and the combined in vivo – in vitro experiments have been done with the same lamp, while the patients were treated with another type of lamp, which makes comparison somewhat uncertain. It seems reasonable to infer from these experiments, however, that the doses of 8-MOP and UVA given to dividing epidermal or dermal cells in the treatment of psoriatic patients are probably close to those which are capable of causing a significant increase in chromosomal aberrations.

The results of the in vivo study neither support nor exclude the possibility that dithranol or 8-MOP + UVA therapy in vivo causes chromosome aberrations in peripheral lymphocytes, especially so as an increased frequency of chromosome aberrations has been reported for lymphocytes of psoriasis patients not receiving systemic treatment when investigated (14). Moreover, there are conflicting reports as to the cause and persistence of chromosome aberrations following treatment with methotrexate and other folic acid inhibitors (17, 24), which have frequently been used also among the psoriasis patients of this study. Thus it is unclear whether the slightly increased mean aberration frequency recorded in the psoriasis groups compared with the control group is due to the disease itself, to treatment, or to combinations of these factors. There are no indications of a "dark" effect on chromosomes by 8-MOP, since the frequency of aberrations in non-irradiated lymphocytes which had been treated with 8-MOP in vivo was within the normal range in all cases (P9–P12).

The 3 patients (D1, D4, P8) who demonstrated a significantly increased frequency of aberrations did not report any previous therapy or exposition which could be taken to explain these results. Case P8 is particularly interesting, since she had not received antipsoriatic treatment before the 8-MOP + UVA treatment, yet she presents with a very high aberration frequency. Therefore the possibility of great individual variations in the spontaneous chromosome aberration frequencies among psoriasis patients, as well as in the response to treatment with regard to cytogenetic effects, should be considered. In future analyses, patients will be investigated before and after treatment for a proper evaluation of the tentative effect of psoriasis therapy on the frequency of chromosome aberrations.

CONCLUSIONS

It is our opinion that orally administered 8-MOP followed by UVA-irradiation gives a good antipsoriatic effect and a good cosmetic result. As yet, we have found no serious side effects. Chromosome aberrations were induced by the treatment in vitro but whether the doses given clinically produce such effects on epidermal or dermal cells could not be decided with certainty. The large number of times each patient probably will be treated over the years certainly demands a careful follow-up of the patients on this therapy.

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REFERENCES

1. Arnold, C. G.: Induction of extracaryotic mutation. *In* Chemical Mutagenesis in Mammals and Man (eds. F. Vogel & G. Röhrborn), pp. 285–293. Springer-Verlag, Berlin, Heidelberg, New York 1970.
2. Baden, H. P., Parrington, J. M., Delhanty, J. D. A. & Pathak, M. A.: DNA synthesis in normal and xeroderma fibroblasts following treatment with 8-methoxypsoralen and longwave ultraviolet light. Biochim Biophys Acta *262:* 247, 1972.
3. Bordin, F., Baccichetti, F. & Musajo, L.: Inhibition of nucleic acid synthesis in Ehrlich ascite tumor cells by irradiation in vitro (365 nm) in the presence of skin-photosensitizing furocoumarins. Experientia *28:* 148, 1972.
4. Court-Drown, W. M.: Human Population Cytogenetics, p. 17, Amsterdam, 1967.
5. Dall'Aqua, F., Marciani, S., Vedaldi, D. & Rodighiero, G.: Studies on the photoreactions (365 nm) between DNA and some methylpsoralens. Biochim Biophys Acta *353:* 267, 1974.
6. Dawber, R.: Oral trisoralen therapy in vitiligo. Br J Dermatol *83:* 386, 1970.
7. Fischer, T., Juhlin, L., Claesson, S. & Alsins, J.: UV-ljusbehandling av psoriasis. Förhandlingar vid Nordisk Dermatologisk Förenings 20:e möte, Stockholm 5–8 juni 1974 (eds. N. Thyresson & Ö. Hägermark), pp. 62–63. Almqvist & Wiksell, Uppsala, 1974.
8. Funes-Cravioto, F., Lambert, B., Lindsten, J., Ehrenberg, L., Natarjan, A. T. & Osterman-Golkar, S.: Chromosome aberrations in workers exposed to vinyl chloride. Lancet *1:* 459, 1975.
9. Gillberg, B. O., Zetterberg, G. & Swanbeck, G.: Petite mutants induced in yeast by dithranol, an important therapeutic agent against psoriasis. Nature *214:* 415, 1967.
10. Howard-Flanders, P.: DNA-repair and recombination. Br Med Bull *29:* 226, 1973.
11. Lerner, A. B., Denton, C. R. & Fitzpatrick, T. B.: Clinical and experimental studies with 8-methoxypsoralen in vitiligo. J Invest Dermatol *20:* 299, 1953.
12. Musajo, L., Rodighiero, G. & Dall'Aqua, F.: Evidences of a photoreaction of the photosensitizing furocoumarins with DNA and with pyrimidine nucleosides and nucleotides. Experientia *21:* 24, 1965.
13. Musajo, L., Bordin, F. & Bevilacqua, R.: Photoreaction at 3655 Å linking the 3,4-double bond of furocoumarins with pyrimidine bases. Photochem Photobiol *6:* 927, 1967.
14. Nielsen, J. & Zachariae, H.: Chromosome aberrations in severe psoriasis. Acta Dermatovener (Stockholm) *53:* 192, 1973.
15. Oddoze, L., Témime, R., Marchand, J-P. & Benne, M.: L'association méladine per os et rayons UV dans le traitement du psoriasis. (Note preliminaire.) Bull Soc Franc Derm Syph *74:* 609, 1967.
16. Parrish, J. A., Fitzpatrick, T. B., Tannenbaum, L. & Pathak, M. A.: Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. N Engl J Med *291:* 1207, 1974.
17. Ryan, T. J., Boddington, M. M. & Spriggs, A. I.: Chromosomal abnormalities produced by folic acid antagonists. Br J Dermatol *77:* 541, 1965.
18. Sasaki, M. S. & Tonomura, A.: A high susceptibility of Fanconi's anemia to chromosome breakage by DNA cross-linking agents. Cancer Res *33:* 1829, 1973.
19. Swanbeck, G. & Zetterberg, G.: Induction of respiration-deficient yeast by methotrexate and its relevance for the antipsoriatic effect. Acta Dermatovener (Stockholm) *53:* 465, 1973.
20. Swanbeck, G. & Thyresson, M.: Induction of respiration-deficient mutants in yeast by psoralen and light. J Invest Dermatol *63:* 242, 1974.
21. Tronnier, H. & Schule, D.: First results of therapy with longwave UV after photosensitization of skin. *In* Schenk (ed): Book of Abstracts, Symposia, and Contributed Papers, Sixth Intern Congr Photobiol, Bochum, Germany p. 340.
22. — Zur dermatologischen Therapie von Dermatosen mit langweiligem UV nach Photosensibilisierung der Haut mit Methoxsalen. Erste Ergebnisse bei der Psoriasis vulgaris. Z Hautkr *48:* 385, 1973.
23. Verbov, J.: Oral trisoralen in psoriasis. Br J Dermatol *88:* 518, 1973.
24. Voorhees, J. J., Janzen, M. K., Karrel, R. E. & Chakrabarti, S. G.: Cytogenetic evaluation of methotrexate-treated psoriatic patients. Arch Dermatol *100:* 269, 1969.
25. Walter, J. F. & Voorhees, J. J.: Psoriasis improved by psoralen plus black light. Acta Dermatovener (Stockholm) *53:* 469, 1973.
26. Weber, G.: Combined 8-methoxypsoralen and black light therapy of psoriasis. Br J Dermatol *90:* 317, 1974.
27. Zetterberg, G. & Swanbeck, G.: Studies on dithranol and dithranol-like compounds. II. Mutagenicity. Acta Dermatovener (Stockholm) *51:* 45, 1971.

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G. Swanbeck, M.D.
Department of Dermatology
Karolinska sjukhuset
S-104 01 Stockholm 60
Sweden