

SISTER CHROMATID EXCHANGES IN LYMPHOCYTES FROM PSORIASIS PATIENTS TREATED WITH 8-METHOXYPsorALEN AND LONGWAVE ULTRAVIOLET LIGHT

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Abstract. The frequency of sister chromatid exchanges (SCE) was analysed in peripheral lymphocytes from patients receiving 8-methoxypsoralen (8-MOP) and long-wave ultraviolet light (UVA) against psoriasis. The average SCE frequency in 14 patients after 3-10 weeks of clinical treatment did not differ statistically from that before treatment. UVA irradiation in vitro of blood from patients receiving 8-MOP caused a significant increase in the number of SCE. Such an increase was not observed after systemic administration of 8-MOP excluding UVA irradiation in vitro, nor after UVA irradiation in vitro in the absence of 8-MOP. Thus, the combination of oral 8-MOP administration and subsequent UVA irradiation in vitro promotes SCE in peripheral lymphocytes. However, this effect is not seen after clinical irradiation to the skin in vivo. The reason for this may be that the accumulated dose of UVA light in circulating lymphocytes, even after several weeks of clinical therapy, is not large enough to produce a significant amount of DNA damage leading to SCE, or else that DNA damage caused by the treatment is efficiency repaired during the intervals between treatments.

Key words: Psoriasis; Ultraviolet light; Psoralens; Sister chromatid exchanges

PUVA treatment, i.e. orally administered 8-methoxypsoralen (8-MOP) followed by exposure of the skin to longwave ultraviolet light (UVA light) has recently been introduced as an effective therapy against psoriasis (9, 13, 16). Concern has been expressed, however, regarding a possible mutagenic and carcinogenic effect of the treatment (12, 14, 16). 8-methoxypsoralen photoreacts with DNA when irradiated at 365 nm in vitro (4) and in vivo (3). The photochemical reaction produces monofunctional adducts with pyrimidine bases and interstrand cross-links. PUVA treatment of human lymphocytes in vitro causes a significant, dose-related increase in chromosome aberrations (13) and sister

chromatid exchanges (SCE) (2). SCE are sensitive indicators of chromosome damage caused by various alkylating agents and cytotoxic drugs (11). They arise by exchange of homologous chromatid segments within chromosomes, probably as a result of double strand breaks in DNA (Fig. 1). In the present work we have studied the frequency of sister chromatid exchanges in lymphocytes from psoriasis patients, before, during, and after PUVA-treatment.

MATERIAL AND METHODS

Patients

Patients who were resistant to conventional psoriasis treatment were selected. They had not received any type of medical treatment during one month prior to investigation. The treatment schedule was as described previously (13), i.e. each patient received 0.6 mg/kg of 8-MOP (Neo meladine, The Memphis Chemical Co, Cairo, Egypt) orally and 2.0-2.5 hours later irradiation of the body was initiated using black light fluorescent lamps (Philips TL 40/08) with a spectral maximum at 360 nm. The irradiation time was generally 20 minutes, delivering a total dose of about 3 J/cm². The treatment was repeated 5 days a week for a number of weeks, depending on the progress of healing, after which maintenance treatment was started (13).

In vivo studies

A blood sample taken immediately before commencement of the treatment schedule served as control. Another blood sample was taken when the patients were healed, which varied from 3-30 weeks of treatment. The lymphocytes were cultured in the dark for 75 hours in the presence of 100 µM bromodeoxyuridine (BrdU) (7), and SCE were studied by the fluorescent plus Giemsa (FPG) technique (7, 10). All cultures and slides were coded so that the persons who handled the cultures and carried out the SCE analyses could distinguish neither between patients, nor between different samples from the same patient. The

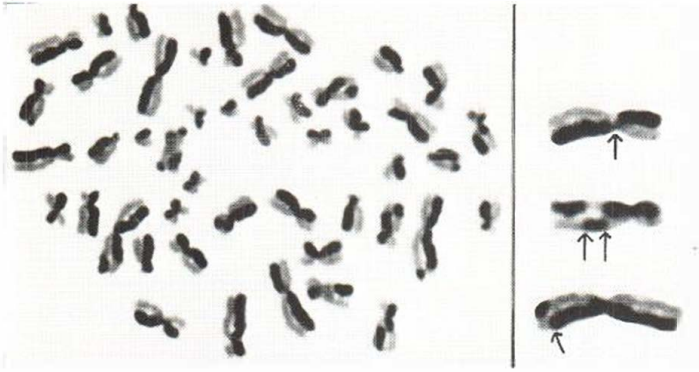


Fig. 1. Human metaphase chromosomes with differentially stained chromatids. Sister chromatid exchanges (SCE) are visible as exchanges of lightly and darkly stained segments of the two chromatids within a number of chromosomes. The inset shows enlarged micrographs of three chromosomes displaying (in order from the top): centromeric SCE, interstitial SCE and terminal SCE. The number of SCE counted in each case are indicated by the arrows.

number of SCE in 20 randomly selected cells from each patient before and after treatment was counted.

Combined in vivo - in vitro studies

Blood samples were taken from psoriasis patients on the first day of treatment, 2 hours after ingestion of 8-MOP. The blood was UVA irradiated in vitro using the same light sources as for clinical irradiation. Two different UVA doses of therapeutic magnitude were given, 1.8 J/cm² and 3.6 J/m². The cell cultures and slides were prepared as described above.

RESULTS

Fourteen patients were studied before and after treatment. The results, expressed as the average number of SCE per cell for each patient, are shown

in Table I. The mean value for the group of patients before treatment did not differ statistically from that after treatment. No evidence was obtained that the duration of PUVA therapy influences the number of SCE.

The results from the combined in vivo - in vitro experiments are shown in Table II. The SCE frequencies in non-irradiated cells from 8-MOP patients did not differ significantly from the SCE frequencies of the control patients receiving no 8-MOP (Table II), nor from the SCE frequencies recorded for patients before or after PUVA therapy (Table I).

UVA irradiation in vitro of cells from patients who had taken 8-MOP 2-2.5 hr before blood sampling caused an increase in the SCE-frequency (Table II). The lower UVA dose (1.8 J/cm²) in-

Table 1. Frequencies of SCE in lymphocytes from patients with psoriasis before and after intensive PUVA-treatment

Patient	Age (years)	Sex	Duration of intensive therapy (weeks)	Number of SCE/cell (mean of 20 cells)	
				Before treatment	After treatment
BB	52	♀	3	16.9	18.4
PO	14	♀	7	12.7	8.8
KK	62	♀	9	20.0	21.1
DB	30	♂	10	18.3	11.5
BD	63	♀	11	17.5	22.8
AH	29	♂	15	16.5	13.6
KS	23	♀	16	12.4	10.9
GH	32	♂	18	15.0	12.5
KH	23	♂	19	17.1	15.9
BL	35	♀	19	15.5	17.0
JS	17	♂	21	13.9	10.4
UL	35	♂	27	13.6	13.2
AE	29	♀	28	18.1	12.6
BA	50	♂	30	13.0	10.1
Mean ± S.D. (n = 14)				15.8 ± 2.4	14.2 ± 4.3

Table II. Frequencies of SCE in UVA-irradiated lymphocytes from psoriasis patients 2 hours after oral administration of 8-MOP and from control patients receiving no 8-MOP

	Number of SCE/cell (mean of 20 cells)		
	No irradiation	UVA-irradiated	
		1.8 J/cm ²	3.6 J/cm ²
<i>Patients receiving 8-MOP</i>			
GH	16.6	15.0	21.4
BD	11.2	23.0	24.2
KS	13.2	16.7	25.4
BL	23.7	23.3	28.9
JS	17.2	19.0	22.8
AH	17.4	26.9	31.6
AE	16.8	24.1	18.4
KK	16.7	21.4	26.4
DB	18.7	24.5	28.7
BA	18.4	24.5	30.0
BB	18.1	21.3	27.7
UL	13.4	16.8	16.7
PO	16.3	29.3	34.8
EK	24.0	30.0	29.8
ÖO	14.4	18.4	18.6
MJ	23.2	29.2	40.2
KW	12.6	20.9	22.9
Mean±S.D. (n=17)	17.2±3.8	22.6±4.6	26.4±6.1
<i>Control patients</i>			
CK	19.3	10.6	15.1
KP	19.8	18.7	30.1
BH	13.4	10.0	11.3
ÅB	15.6	15.4	14.6
EB	11.7	10.5	13.8
Mean±S.D. (n=5)	16.0±3.6	13.0±3.8	17.0±7.5

creased the average frequency of SCE/cell by 24% and the higher UVA dose (3.6 J/cm²) by 54%, compared with non-irradiated cells. These increases are statistically significant ($P < 0.001$ according to analyses of variance). It is worth noting that the higher UVA dose caused an increase in the SCE frequency in all patients, and that the lower dose had the same effect in all but 2 patients (Table II).

UVA-irradiated cells from control patients not receiving 8-MOP displayed SCE frequencies which were not statistically different from those of non-irradiated cells from the same subjects (Table II).

DISCUSSION

Psoriasis is a disease of imperfect keratinization associated with an accelerated turnover of epidermal cells. The therapeutic targets for the clinical

PUVA-treatment are the proliferating cells of the psoriatic epidermis. It is likely that the positive clinical effect is partly the result of cytogenetic damage inhibiting the epidermal cell proliferation (15). Although this effect of the therapeutic regimen is beneficial with regard to the skin disease, it would be hazardous if it were to give rise to chromosome damage in other, proliferating cells. We have shown previously that the combined exposure of human lymphocytes to 8-MOP and UVA light in therapeutic doses does give rise to chromosome aberrations *in vitro*, but we did not observe this effect after *in vivo* exposure of psoriatic patients (13).

Sister chromatid exchanges are probably a much more sensitive indicator of certain types of cytogenetic damage than the conventional chromosome analysis (11, 17). Our present results from the combined *in vivo* - *in vitro* studies clearly demonstrate that PUVA treatment is capable of causing DNA damage which promote SCE in human lymphocytes. Cells exposed to either 8-MOP or UVA light do not exhibit an increased number of SCE; thus it is only the combined exposure of the agents which induces SCE.

The data also imply that the concentration of 8-MOP in serum 2-2½ hours after oral intake of the drug is sufficient to promote SCE in UVA-irradiated lymphocytes. Carter et al. (2) recently presented data on the SCE-promoting effect of PUVA treatment of human lymphocytes *in vitro*. Their results are very similar to our results from the combined *in vivo* - *in vitro* experiments. In fact, on comparing the SCE frequencies induced by the different 8-MOP and UVA doses in the experiments of Carter et al. (2), we may calculate that the serum concentration of 8-MOP in patients 2-2½ hours after oral intake of 0.6 mg/kg is in the range of 10^{-7} - 10^{-8} M.

Although these data clearly demonstrate that PUVA treatment may promote SCE under experimental conditions *in vitro*, we found no evidence of such an effect in the circulating lymphocytes from patients receiving clinical PUVA therapy. The mean frequency of SCE in the group of patients before treatment was not statistically different from that after 3-30 weeks of intensive PUVA treatment (Table I). The recorded SCE frequencies for all patients before as well as after treatment fall within the range for the "spontaneous" frequencies of SCE in lymphocytes from normal subjects reported previously (5-8).

Obviously, the penetration of UVA light is largely limited to the skin, and it is very likely that the accumulated dose of UVA light in the individual circulating lymphocyte, even after several weeks of intensive treatment, is still not high enough to produce a significant amount of DNA damage leading to SCE. Since it has been reported that DNA damage caused by 8-MOP and UVA light is subject to cellular repair processes (1), it is also possible that PUVA-induced DNA damage is efficiently repaired during the intervals between treatments.

Thus, our present results indicate that clinical PUVA treatment does not induce SCE in circulating lymphocytes. It cannot be entirely excluded, however, that cytogenetic damage may occur in other dermal or epidermal cells, since PUVA treatment is obviously capable of producing SCE. We therefore suggest that the clinical PUVA dose at each treatment should be kept low, and that as much time as possible between treatments should be allowed for DNA repair.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Psoriasis Association, the Swedish Work Environmental Fund, and Karolinska Institutet. We are grateful to Öje Laidna and Barbro Werelius for technical assistance.

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Received February 17, 1977

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