ELSEVIER

Mutation Research 312 (1994) 79–83

MR
Environmental Mutagenesis

Mutagenic risk in psoriatic patients before and after 8-methoxypsoralen and long-wave ultraviolet radiation

S. Şardaş [a,*], B. Karahalil [a], A.E. Karakaya [a], R. Şaşmaz [b]

[a] *Gazi University, Faculty of Pharmacy, Toxicology Department, 06330 Ankara, Turkey*
[b] *Social Insurance Ankara Hospital, Clinics of Dermatology, Ankara, Turkey*

(Received 22 July 1993)
(Revision received 26 October 1993)
(Received 10 November 1993)

Abstract

Sister-chromatid exchange (SCE) analysis was carried out in different age groups prior to and after therapy with 8-Methoxypsoralen (8-MOP) followed by exposure of the patient to long-wave UV-A (PUVA) and compared to control. The SCE frequencies were increased significantly in PUVA-treated patients as compared to their pre-treatment SCE levels and to controls. A significant increase in SCEs was found in smoking PUVA-treated patients as compared to non-smoking PUVA-treated patients. This study indicates a detectable chromosome-damaging effect of PUVA therapy on its human users.

Key words: Psoriasis; Ultraviolet light; Sister-chromatid exchanges; PUVA therapy

1. Introduction

8-Methoxypsoralen (8-MOP) and long-wave UV-A light (PUVA) therapy which has been used as an effective therapy against psoriasis and vitiligo leads to a possible mutagenic and carcinogenic effect. 8-MOP photoreacts with DNA when irradiated in vitro and in vivo (Brogger et al., 1978; Bishop, 1979; Bioulac et al., 1980; Bredberg, 1982; Abel, 1987). Photochemotherapy consisting of the administration of 8-MOP followed by exposure of the patient to PUVA has often been found to result in somatic chromosome aberrations in adult patients (Bridges, 1979; Wolff-Schreiner et al., 1977; Bredberg et al., 1983). Since ancient times natural sources of psoralen have been known to promote cutaneous pigmentation (Anderson and Voorhees, 1980). Psoralen photosensitization causes covalent binding of psoralens to pyrimidine moieties in DNA (Scott et al., 1976). Binding requires long-wave UV light (UVA) and both monofunctional psoralen–DNA photoadducts and bifunctional, or cross-linking psoralen–DNA photoadducts affect several important cell functions (Gupta et al., 1987). Cultured human lymphocytes exposed to these agents in vitro show an increased frequency of sister-chromatid exchanges (SCE) related to 8-MOP–DNA photoadducts (Brogger et al.,

* Corresponding author.

SSDI 0165-1161(93)E0048-F

1978). It has also been shown that 8-MOP can also be activated with visible light (419 nm) (Gasparro et al., 1993). In their report bovine aorta smooth muscle cells were treated with 8-MOP and 419-nm light. The primary effect of using visible light instead of long-wavelength ultraviolet radiation is a more than 10-fold reduction in the extent of cross-link formation. The SCE technique, a sensitive indicator of mutagenicity, was used to observe the mutagenic susceptibility in 32 psoriatic patients in different age groups admitted to the Social Insurance Ankara Hospital, Clinics of Dermatology prior to and after PUVA therapy and compared to controls.

2. Materials and methods

2.1. Patients

Peripheral blood was obtained aseptically from 46 untreated psoriatic patients (21 female, 25

Table 1
Clinical data of psoriatic patients, 8-Methoxypsoralen and long-wave ultraviolet light

Patient	Age	Smoking habit	Irradiation (J)	Intensive therapy (weeks)	Mean SCE	
					Before PUVA treatment	After PUVA treatment
AH	18	–	114	30	4.1	6.0
GMS	39	+	111.5	28	5.8	8.9
RK	36	+	118	31	5.7	6.8
FY	34	+	254	60	6.0	6.7
OE	45	+	268	56	6.2	7.0
TD	38	+	142	38	4.8	7.0
TD	29	+	103	58	4.8	6.9
EC	28	+	152.5	48	5.6	7.5
HO	30	+	195	32	7.0	9.0
ÖÇ	43	+	208	62	6.0	7.8
SE	31	+	199	61	5.0	7.1
SA	25	+	132	61	5.1	6.2
CA	43	–	144	56	3.2	6.1
SK	35	+	136	33	4.6	6.6
AK	26	–	133.5	60	3.6	5.6
HD	32	+	80	74	6.2	7.1
EÖ	12	–	145	35	4.1	6.0
MA	40	–	155	44	3.0	6.0
SA	16	+	180	40	4.3	6.9
YT	52	–	252	36	4.0	6.7
FE	60	–	96	20	4.0	8.0
AÇ	18	+	314	28	4.9	7.8
AA	35	–	228.5	30	3.5	4.8
HT	35	+	252	32	5.1	7.3
SA	36	–	230	32	4.2	6.3
MO	42	+	226	23	4.9	6.5
PÇ	42	–	142	32	4.9	8.1
AW	29	–	142	32	4.2	5.8
MA	56	+	150	45	5.0	5.9
NA	60	–	124	30	4.9	6.0
SE	40	+	121	20	4.8	7.0
MG	47	–	120	20	3.2	6.0

+, Smoking 1–20 cigarettes.
8-MOP 0.6–0.9 mg/kg.
Every treatment consisted of 0.5–7.0 J.

male) between the ages of 12 and 65 and only 32 of these patients underwent PUVA treatment (14 female, 18 male). Also blood was obtained from 50 normal healthy subjects (23 male, 27 female) who served as controls and none of them had PUVA treatment at any time. In addition, all subjects were given a detailed questionnaire to provide as much information as possible about factors that may potentially confound the analysis of SCE. Smoking is perhaps the most important confounding factor in the interpretation of SCE frequencies. Therefore we felt that it is important to analyze the data for smoking habits also since 19 of the treated subjects were heavy smokers. The treatment schedule for each patient is described in Table 1.

2.2. Sister-chromatid exchange

Peripheral blood samples were taken from each subject before commencement of the treatment schedule and another blood sample was taken when the patients were healed which varied from 20 to 74 weeks of treatment and blood samples were placed in culture the same day. The culture medium contained TC 199 medium (Seromed) supplemented with 20% fetal calf serum (Seromed), 2.5 ml phytohemagglutinin (Sigma) and 10 μg/ml 5-bromo-2-deoxyuridine (Sigma). The lymphocytes were allowed to divide for two cell cycles at 37°C. During the last 2 h of incubation 0.5 mg/ml colchicine (Sigma) was added to the culture. Chromosomal preparations were made by the conventional method of Wolff and Perry (1974). An average of 20 metaphase plates with 46 intact chromosomes and well-differentiated sister chromatids were scored from each case to calculate mean SCE frequency. All cultures and slides were coded so that the person who handled the cultures and carried out the SCE analyses could distinguish neither between patients and controls, nor between different samples from the same patient. The data concerning the mean numbers of SCE, age prior to therapy, duration of therapy, and cigarette consumption were evaluated with Student's *t*-test.

Table 2
Mean number of SCEs in lymphocytes of psoriatic patients and control subjects

	Number of subject	Mean SCE/cell ± SD
Psoriatic patients		
Prior to PUVA treatment	32	4.81 ± 0.98
Non-smokers	13	3.92 ± 0.64
Smokers	19	5.42 ± 0.69
After PUVA treatment	32	6.84 ± 0.95
Non-smokers	13	6.23 ± 0.92
Smokers	19	7.05 ± 1.02
Healthy donors (controls)	50	5.10 ± 1.65
Non-smokers	27	4.11 ± 0.94
Smokers	23	6.52 ± 1.47

The differences between the groups are statistically significant by Student's *t*-test ($p < 0.001$).

3. Results and discussion

Table 1 shows the clinical data of the psoriatic subjects on PUVA therapy. Thirty-two patients were studied before and after treatment. Each patient received 0.6–0.9 mg/kg of 8-MOP (Neomeladine, Memphis Chemical Co., Cairo, Egypt) orally and the irradiation time was generally 15–20 min, delivering a total dose of about 0.5–7.0 J. The treatment was repeated 3 days a week depending on the progress of healing, after which maintenance treatment was started (Swanbeck et al., 1975). Smoking habits of psoriatic patients were also evaluated. Table 2 shows the mean number of SCEs/cell, and the standard deviation (SD) of the total 32 psoriatic patients prior to PUVA and after PUVA treatment.

The mean SCE frequencies between treated and untreated subjects were significantly higher in PUVA-treated patients ($p < 0.001$). Also the same significance existed between PUVA-treated and control subjects ($p < 0.001$). However, there was no significant difference in mean SCE frequency between untreated patients (4.8 ± 0.98) and controls (5.10 ± 1.65) ($p > 0.05$).

Nineteen of these patients were smokers and their SCE frequencies were highly increased (7.05 ± 1.02) although the 13 non-smoking patients

had a mean value of 6.23 ± 0.92 SCEs after treatment.

Smoking plays a causative role in the higher incidence of SCEs. Increased levels of SCE in peripheral lymphocytes of smokers have been confirmed by various reports (Ghosh and Ghosh, 1978; Sarto et al., 1978; Sardas et al., 1991). No significant relationship was found between age and SCEs. The induction of SCE by photoaddition of a monopsoralen (Pyridopsoralen) and a bifunctional psoralen (8-MOP) in normal and Fanconi anemia fibroblast cell lines was compared by Billardon and Moustacchi (1986); normal cells demonstrated a higher sensitivity to photoaddition of the monofunctional psoralen used than to 8-MOP. These in vitro data are likely to be related to the higher amount of lesions induced by the monofunctional psoralen in DNA.

In conclusion, the present study differs from previous SCE studies by Mourelatos et al. (1977) and Lambert et al. (1978) with negative results concerning the SCE test in psoriasis 2 h after oral administration of 8-MOP but before UV-A irradiation either in vivo or in vitro. The recorded mean frequency of SCE in the group of 14 patients by Lambert et al. (1978) before treatment were not statistically different from that after 3–30 weeks of PUVA treatment. However, no comparison could be made with previous reports since our patients had had 20–74 weeks of intensive PUVA treatment and it is also possible that PUVA-induced DNA damage is efficiently repaired depending on the intervals between treatments. 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.

4. References

Abel, G. (1987) Chromosome damage induced in human lymphocytes by 5-Methoxypsoralen and 8-Methoxypsoralen plus UV-A, Mutation Res., 190, 63–68.

Anderson, T.F., and J.J. Voorhees (1980) Psoralen photochemotherapy of cutaneous disorders, Pharmacol. Toxicol., 20, 235–257.

Billardon, B., and E. Moustacchi (1986) Comparison of the sensitivity of Fanconi's anemia and normal fibroblasts to the induction of sister-chromatid exchanges by photoaddition of mono- and bi-functional psoralens, Mutation Res., 174, 241–246.

Bioulac, P., I. Denechaud, M.S. Doutres, M. Ducassou and C. Beylots (1980) Unscheduled DNA synthesis in psoriatic skin after ultraviolet irradiation and the effects of a combined treatment with 8-Methoxypsoralen and longwave ultraviolet radiation: a clinical study, Br. J. Dermatol., 102, 285–295.

Bishop, S.C. (1979) DNA repair synthesis in human skin exposed to ultraviolet radiation used in PUVA (Psoralen and UV-A) therapy for psoriasis, Br. J. Dermatol., 101, 399–405.

Bredberg, A. (1982) Genetic toxicity of psoralen and ultraviolet radiation in human cells, Acta Dermatol. Venereol., 104, 1–40.

Bredberg, A., B. Lambert, A. Lindblad, G. Swanbeck and G. Wennersten (1983) Studies of DNA chromosome damage in skin fibroblasts and blood lymphocytes from psoriasis patients treated with 8-methoxypsoralen and UV-A irradiation, J. Invest. Dermatol., 81, 93–97.

Bridges, B.A. (1979) An estimate of genetic risk from 8-Methoxypsoralen photochemotherapy, Hum. Genet., 49, 91–96.

Brogger, A., H. Wakswik and P. Thune (1978) Psoralen/UV-A treatment and chromosomes, Arch. Dermatol. Res., 261, 287–294.

Gasparro, F.P., P. Gattolin, G.A. Olack, L.I. Deckelbaum and B.E. Sumpio (1993) The excitation of 8-methoxypsoralen with visible light; reversed phase HPLC quantitation of monoadducts and cross-links, Photochem. Photobiol., 57, 1007–1010.

Ghosh, R., and P.K. Ghosh (1987) The effect of tobacco smoking on the frequency of sister chromatid exchanges in human lymphocytes chromosomes, Cancer Genet. Cytogenet., 27, 15–19.

Gupta, A.K., and T.F. Anderson (1987) Psoralen Photochemotherapy, Mutation Res., 17, 703–735.

Lambert, B., M. Morad, A. Bredberg, G. Swanbeck and M. Thyresson-Hök (1978) Sister chromatid exchanges in lymphocytes from psoriasis patients treated with 8-methoxypsoralen and longwave ultraviolet light, Acta Dermatovenereol. (Stockholm), 58, 13–16.

Mourelatos, D., M.J.W. Faed, P.W. Gould, B.E. Johnson and W. Frain-Bell (1977) Sister chromatid exchanges in lymphocytes of psoriatic after treatment with 8-methoxypsoralen and long wave ultraviolet radiation, Br. J. Dermatol., 97, 649–655.

Sarto, F., L. Mustari, D. Mazzotti, R. Tomanin and A.G. Levis (1987) Variation of SCE frequencies in peripheral lymphocytes of ex-smokers, Mutation Res., 192, 157–162.

Scott, B.R., M.A. Pathak and G.R. Mohn (1976) Molecular and genetic basis of furocoumarin reactions, Mutation Res., 39, 29–74.

Swanbeck, G., M. Thyresson-Hök, A. Bredberg and B. Lambert (1975) Treatment of psoriasis with oral psoralens and long wave ultraviolet light; therapeutic results and cytogenetic hazards, Acta Dermatovenereol. (Stockholm), 55, 367.

Şardaş, S., S. Gök and A.E. Karakaya (1991) Increased frequency of sister chromatid exchanges in the peripheral lymphocytes of cigarette smokers, Toxicol. In Vitro, 5, 263–265.

Wolff-Schreiner, E.C., D.M. Carter, H.G. Schwarzacher and K. Wolff (1977) Sister chromatid exchanges in photochemotherapy, J. Invest. Dermatol., 69, 387–391.