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Micronucleus Evaluation in Mitogen-Stimulated Lymphocytes of PUVA Treated Patients

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HAMURCU, Z., DEMİRTAS, H., ASCIOGLU, Ö., DÖNMEZ-ALTUNTAS, H. and AKTAS, E. *Micronucleus Evaluation in Mitogen-Stimulated Lymphocytes of PUVA Treated Patients.* Tohoku J. Exp. Med., 2002, **198** (1), 11-21 —— PUVA describes the treatment of patients with psoralens plus an exposure to a source of UV light of 320-400 nm (UVA). Contradictory results have been reported on the chromosomal damage of PUVA when assayed by sister chromatid exchange (SCE) method. Micronucleus (MN) test is used to detect both clastogenic (breaking) and aneugenic (abnormal segregation) effect of physical/chemical agents on the chromosomes. No data have been found on the MN formation in the cells of PUVA treated patients. Frequency of micronuclei in 72 hours cultivated/mitogen-stimulated lymphocytes of patients have been evaluated at zero time and after 20, 40, 60 sessions of PUVA treatment. While the beginning MN frequency was $\sim$0.22% ($n=23$), it raised to $\sim$0.32 ($n=23$), $\sim$0.42 ($n=14$) and $\sim$0.53% ($n=10$) corresponding respectively to 20, 40 and 60 sessions. These sessions correspond reciprocally to 54 ± 23, 172 ± 48, 300 ± 61 joules/cm^2 of UVA and 13, 26, 39 mg/kg of 8-metoxypsoralen (8-MOP). While large interindividual variances were apparent, highly significant differences have been observed between initial MN frequency and after that of the 20, 40 and 60 sessions, ($p=0.000$, $p=0.004$, $p=0.005$, reciprocally, Wilcoxon two-related samples test). The coefficient of correlation between MN frequency and UVA doses starting from zero to 60 sessions of treatment has been found as $r=0.61$. This indicates a significant relationship between UVA doses and MN frequencies. However, MN inducibility and synergistic property of 8-MOP with UVA should be taken into account. Gradual MN increase during different sessions of PUVA treatment shows that -once appeared-, a part of MN at least persist in the cells of patients from a few days to a few weeks. Smoking as a confounding factor seems to increase MN frequency ($p=0.053$, Mann-Whitney U-test) in the beginning population, taken as the control population. This is the first report on the kinetics of MN formation during different sessions of PUVA treatment. Based on our results, we concluded that PUVA treatment causes a detectable chromosome damaging effect on the relatively

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profound cells/tissues of its human users. Therapists should be careful with its use, especially on the patients who may be more susceptible to carcinogenesis (e.g. immunosuppressed and/or elderly subjects). ———— PUVA; micronucleus; psoriasis; vitiligo; alopecia areata.
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The term PUVA (Psoralens plus ultraviolet A) describes a form of treatment, embracing psoralens, a group of furocoumarin compounds and exposure of the patients to a source of UV light of 320-400 nm, used for several years as an effective photochemotherapy of some human skin disease such as psoriasis and vitiligo (Baker and Wilkinson 1979; Parrish et al. 1982; Knobler et al. 1988). This therapy is also used for other disorders such as the generalised cutaneous leukaemic cell infiltration (Takemori et al. 1995), anaplastic cutaneous CD30+T-cell lymphoma (Wickenhauser et al. 1999), mycosis fungoides (Takeoka et al. 2000), cutaneous T-cell lymphoma and lymphomatoid papulosis (Nevala et al. 2001).

PUVA treatment leads also to mutagenic and a possible carcinogenic effect (Bredberg et al. 1983; Filipe et al. 1997; Stern et al. 1998; Cooke et al. 2001). The molecular mechanisms of PUVA-dependent damage (Averbeck et al. 1990; Möller et al. 1995; Gasparro et al. 1997; Cooke et al. 2001; Mori et al. 2001), its repair (Averbeck et al. 1990; Mori et al. 2001) and UV damage in general (Otrin et al. 1997) have been investigated. Formation of UV photoproduct (Pfeifer 1997) and in vitro photogenotoxicity testing (Meunier et al. 2002) have been reviewed.

Interestingly, no data have yet been published on the in vivo induction of micronucleus in the lymphocytes of PUVA treated patients. Whereas micronucleus (MN) assay is currently used to detect and quantify phototoxicity and photogenotoxicity (Snyder and Cooper 1999). Furthermore, MN test is recognized as the one only being able to detect both clastogenic (breaking) and aneugenic (abnormal segregation) events (Krishna and Hayashi 2000; Meunier 2002). Moreover, photomicronucleus test has been recommended for photochemical genotoxicity assessment in the IWGTP workshop held in Washington in 1999 (Gocke et al. 2000).

Micronuclei are formed by chromosomal damage or spindle poison when cells divide. Chromosomal fragments or entire chromosomes with centromere (mediated by a spindle poison appeared in the cell through a physical or chemical mutagen) lag behind and are excluded from the main nuclei in the daughter cells. These fragments or whole chromosomes form their own membranes and appear as Feulgen-specific bodies termed micronuclei in the cell cytoplasm (Rosin 1992). In the other experimental system, MN formed after the action of a test agent in polychromatic/ normochromatic erythrocyte (when the main nucleus is extruded) is evaluated (Krishna and Hayashi 2000). Since MN are formed in dividing cells, it is necessary to use cytochalasin B if one want to evaluate only newly formed MN in nucleated cells in a limited time (e.g. 24 or 48 hours), just after the action of a given genotoxin.

UV radiation may damage DNA in one of the two ways: either directly, leading to lesions such as cyclobutane pyrimidine dimmers, pyrimidine-pyrimidone (6-4) photoproducts or indirectly, via photosensitizers (e.g., 8-methoxypsoralens [8-MOP]) that generate free radical species that may ultimately produce such oxidative lesion as 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Ahmed et al. 1999; Cooke et al. 2001). 8-OHdG formation was correlated with both UVA fluence and 8-OHdG concentration (Liu et al. 1999).

Apoptotic degeneration of the epidermal

cells after PUVA treatment has been reported firstly by Hashimoto and Kumakiri (1979). Subsequent studies established that PUVA treatment induces apoptosis of infiltrating inflammatory cells that are responsible for the formation of psoriatic lesions (Enomoto et al. 1997; Coven et al. 1999; Laporte et al. 2000). In fact PUVA was used as an effective therapy via apoptosis for cutaneous diseases caused by skin infiltration with normal or neoplastic T-lymphocytes (Takemori et al. 1995; Johnson et al. 1996; Yoo et al. 1996; Morita et al. 1997).

There are different methods to assess genotoxicity of a given compound or a physical agent. MN test is a more practical technique compared with metaphase analysis and other sophisticated methods (for a review see Tice et al. 2000) if one simply wants to know whether chromosome breakage or loss is being induced (Heddle 1973; Fenech 1997). The MN test was devised primarily for screening chemicals for in vivo chromosome-breaking effect (Heddle 1973; Schmid 1975). Because of its simplicity, sensitivity and its ability to detect both clastogens and aneuploidy-inducing agents, the MN test has been widely used to measure genotoxicity both in vivo and in vitro (for the recent evaluation see Kirsch-Volders et al. [2000] and Krishna and Hayashi [2000]). Another cytological method to detect chromosomal damage is sister chromatid exchange (SCE) and using this method, contradictory results were reported on the effect of PUVA: Mourelatos et al. (1977) and Lambert et al. (1978) have reported that PUVA do not induce SCE in lymphocytes of treated patients. However, Sardas et al. (1994) have reported the contrary results. In the following we report dose (UVA+8-MOP)-related increase in MN frequency in lymphocytes of PUVA treated patients.

Materials and Methods

Patients

23 patients between the age of 13 and 52 resistant to conventional treatment (14 female, 9 male) were selected for the PUVA treatment. Nine of them were diagnosed as psoriasis, 8 as vitiligo and 6 as alopesi areata. A questionnaire collecting information on the past medical history, alcohol, drug and smoking habits, was completed for each patient. Eight patients after 20 sessions and 6 after 40 sessions have abandoned the treatment. Because they have not returned for the control, their remission status have not been evaluated. 8-MOP 0.6-0.7 mg/kg was given orally at the beginning of each session. Two hours later, UVA irradiation of the body surface was followed by a cabin Waldman UV 8001 K. At the beginning, patients have received 0.5 joules (J) UVA/cm^2. This dose was increased regularly 0.5 J after each of 3 sessions of treatment. The treatment was repeated 3-4 days a week until the maintenance therapy was started. Maintenance therapy was administered once or twice a week (7 J or 14 J/cm^2) for an average of 4 months or more. If the erythema had appeared during the treatment, the dose would have been decreased or not increased according the skin type and the clinical state of the patient.

Micronuclei

Peripheral blood was obtained aseptically from patients at zero time and after different sessions of treatment. 0.3 ml whole blood was added to 5 ml Ham's F10 medium (Biological Industry [Biol. Ind.]) enriched with 30% fetal calf serum (Biol. Ind.) 2% v/v phytohemagglutinin (PHA) (Biol. Ind.), 1% v/v penicillin-streptomycin (Biol. Ind.) and 1% heparin in sterile plastic culture tubes.

Cells were harvested at the end of 72 hours, treated with hypotonic solution 0.1 M KCl for 4-5 minutes and fixed in two changes of methanol-acetic acid (3 : 1). Spreading on the slide, drying and Giemsa staining were followed. For each individual 2400-6500 PHA-stimulated cells with clearly apparent and preserved cytoplasm were examined for the

presence of the micronuclei. Small non-stimulated cells were not scored. Slides were scanned using 400× magnification and when located, micronucleated cells were analysed with 1000× magnification. Cells with MN were identified according to the criteria of Bush and McMillan (1993).

Statistical analysis

Statistical comparisons on mean values of MN frequency before and after treatment were done using Student's *t*- and Wilcoxon tests. Linear regression analysis was used to assess the relationship between MN frequencies and UVA (J/cm^2) doses. Nonparametric Friedman and Wilcoxon tests were used to evaluate micronuclei frequency in 8 patients who were submitted to all of the PUVA sessions. The effect of smoking on MN induction is tested by Mann-Whitney U-test.

Results

The Table 1 shows the means of MN frequencies of the patients at zero time and after 20, 40 and 60 sessions of treatment. These sessions correspond reciprocally to 54±23, 172±48, 300±61 Joules of UVA and 13, 26, 39 mg/kg of 8-MOP. Fig. 1 shows the dispersion of individual MN frequencies (means) obtained after different session of PUVA treatment. Large interindividual variation concerning MN frequency before and after each session of treatment is apparent. However there was a highly significant increase in means of MN frequencies

Table 1. *MN frequencies of the patients before and after 20, 40, 60 sessions of PUVA treatment in comparison with their pre-treatment values. Statistical comparisons of MN frequencies before and after treatment have been done according to the Wilcoxon test*

20 session Mean dose: 54±23 J/cm²±S.D. Plus 13 mg/kg of 8-MOP	Cell scored (Mean±S.D.)	MN scored (Mean±S.D.)	MN/Cell (%) (Mean±S.D.)
Before treatment* ($n=23$)	3563±603	7.52±3.39	0.22±0.11
After treatment ($n=23$)	3648±951	11.26±4.04	0.32±0.11
			$p=0.000$
40 session **Mean dose: 172±48 J/cm²±S.D.** **Plus 26 mg/kg of 8-MOP**	**Cell scored** **(Mean±S.D.)**	**MN scored** **(Mean±S.D.)**	**MN/Cell (%)** **(Mean±S.D.)**
Before treatment* ($n=14$)	3654±697	7.42±3.27	0.21±0.10
After treatment ($n=14$)	3479±489	14.64±5.71	0.42±0.15
			$p=0.004$
60 session **Mean dose: 300±61 J/cm²±S.D.** **Plus 39 mg/kg of 8-MOP**	**Cell scored** **(Mean±S.D.)**	**MN scored** **(Mean±S.D.)**	**MN/Cell (%)** **(Mean±S.D.)**
Before treatment*($n=10$)	3522±670	8.30±3.59	0.24±0.12
After treatment ($n=10$)	3178±291	17.0±5.35	0.53±0.16
			$p=0.005$

*These means represent the base line means found at the beginning within 23 individuals at zero time without PUVA treatment. They do not represent newly measured values.

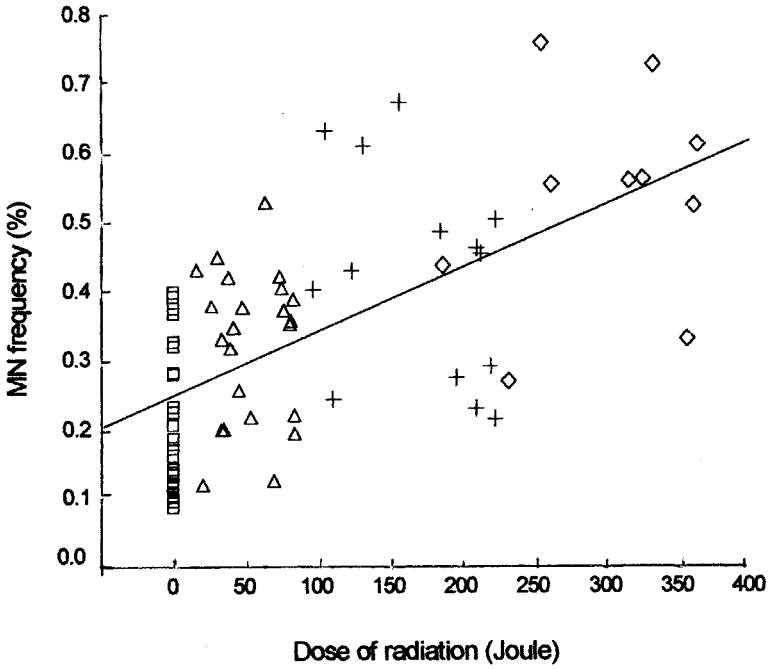


Fig. 1. Dose-related increase in micronuclei frequencies of 72 hours cultured lymphocytes from the PUVA treated patients. Individual MN frequencies are indicated by □, △, + and ◇ correspond, respectively to zero, 20, 40 and 60 sessions of UVA and the same signs indicate the corresponding doses (0, 13, 26 and 39 mg/kg) of 8-MOP (□, 0.00; △, 13.00; +, 26.00; and ◇, 39.00 mg/kg). Rsq=0.3760.

after each session of PUVA treatment (20, 40 and 60 sessions) when compared to their pre-treatment values ($p=0.000$, $p=0.001$, $p=0.001$ respectively by Student's t-test and $p=0.000$, $p=0.004$, $p=0.005$ by Wilcoxon test, Table 1). The coefficient of correlation between MN frequency and UVA doses starting from 20 session to 60 session of treatment has been found as $r=0.48$. The same coefficient, starting from zero time to 60 session has been found as $r=0.61$. This indicates an obvious relationship between UVA doses and induced MN frequencies. In other word, the means of the MN frequencies in each session increase according to the UVA doses. While the beginning MN frequency was ~0.22%, it was risen to ~0.32, ~0.42 and ~0.53% corresponding respectively to 20, 40 and 60 sessions of treatment. However these data should be interpreted with caution because psoralens (here 8-MOP) alone may induce MN and may act synergistically with UVA as it is stated in the "discussion."

The base-line value ($0.22\pm0.11\%$, mean± S.D.) of MN frequency before the treatment of 23 patients is comparable with the previous findings on the two different control populations: ([$0.24\pm0.13\%$, Hamurcu et al. 2001] and [$0.24\pm0.20\%$, Forni 1994]). The similarity of this base-line value with those of the two independent studies on the two independent control populations and the regular MN frequency increase during the different session of PUVA treatment (Table 1 and Fig. 1) indicates that our MN testing method is reproducible. Falck et al. (1997) have reported lower base-line MN frequencies in 5 non-smoking donors (41-50 years old) both with and without cytochalasin B in isolated lymphocytes: (~0.14% and ~0.17% respectively). However their cases number is insufficient to compare with our result and to evaluate the effect of lymphocyte purification on the MN content. It may be more informative to compare the intersession MN content of patients who were submitted to

TABLE 2. *Micronucleus frequencies of 8 patients (BG, AK, EI, AU, GC, ZO, YG, HA) who were exposed to all of the 0, 20, 40, 60 sessions of PUVA treatment*

Session	Cell scored (Mean±S.D.)	MN scored (Mean±S.D.)	MN/Cell (%) (Mean±S.D.)	8-MOP mg/kg+UVA (J/cm²) (Mean±S.D.)
Before treatment ($n=8$)	3639.88±699	7.25±3.20	0.20±0.08	
After 20 session ($n=8$)	3447.25±592	12.00±3.66	0.35±0.11	13+ 64.48±15.01
After 40 session ($n=8$)	3482.00±582	15.00±5.21	0.43±0.14	26+175.59±45.46
After 60 session ($n=8$)	3192.88±327	16.25±5.39	0.51±0.16	39+297.33±63.03

all sessions of the PUVA treatment. For this, MN frequencies of 8 patients (AK, AU, BG, EI, GC, HA, YG and ZO, Table 2.) who were exposed to all of the PUVA sessions have been compared by nonparametric Friedman test. Because an increase in the frequency of MN was found significant by this test ($p=0.003$), the comparison MN content of each session with another session has been performed by Wilcoxon two-related samples test. The results of the analysis according to this test were found as the following: Between 0 time and 20 sessions ($p=0.012$), zero time and 40 sessions ($p=0.017$), zero time and 60 sessions ($p=0.012$). However MN frequency was not significantly different between 20 and 40 sessions, 20 and 60 sessions, 40 and 60 sessions, ($p>0.05$).

No significant relationship was found between age and MN frequency ($p>0.05$). However our patients were composed mostly from young individuals (mean value ~25 years old) and except one patient who may be considered as middle-aged (52 H.A.), the elder patients have been completely absent. When compared at zero time between 23 patients (7 smokers against 16 non smokers), smoking seems to enhance MN frequencies to some extent (according to Mann-Whitney U-test $p=0.053$, to Student t-test, $p<0.05$). However the number of cases may be considered few for the t-test. Based on their statement, alcohol consumption as a confounding factor has not been noted in our patients.

DISCUSSION

This study demonstrates that treatment of patients with PUVA results in an increase of MN frequencies in lymphocytes in vivo. Similarly, increased levels of SCE were observed by Sardas et al. (1994) in lymphocytes of psoriatic patients after PUVA treatment. However, others reported that PUVA treatment of the psoriatic patients does not change SCE level in the lymphocytes (Mourelatos et al. 1977; Lambert et al. 1978). Furthermore, Bredberg et al. (1983) have found normal levels of SCE in lymphocytes of psoriatic patients after 3-30 weeks of PUVA treatment. The same authors have observed a small but statistically significant increase of SCE if the duration of PUVA treatment was longer (1-6 years). We do not know why so contradictory results have been reported with the same method with the same cells (lymphocytes) on the same disorder (psoriasis).

Although these two methods (SCE and MN assay) were used for the genotoxicity testing, their mechanisms are different. In fact, the mechanisms of SCE induction by mutagens are not only related with the chromosome aberrations but also related with the repair replication (Thust et al. 1996), inhibitors of DNA synthesis (Pinero et al. 1996), inhibitor of poly (ADP-ribose) polymerase (Schwartz and Weichselbaum 1985; Catena et al. 1994), homologous recombination (Sonoda et al. 1999). For instance, it has been reported that X-ray does not increase SCE in normal lymphocytes

(Andriadze et al. 1987), it is known to be poor inducer of SCEs (Darroudi and Natarajan 1987) whereas X-ray is known as a powerful clastogen and by consequence MN inducer (Catena et al. 1994; Odagiri et al. 1997; He et al. 2000). Some chemicals have been implicated as teratogens and carcinogens but are negative as SCE inducers (Liang et al. 1983).

It has been reported that PUVA leads to chromosome breakage via the formation of transferable clastogenic factors (CF) (Filipe et al. 1997). According to these authors, superoxide generated via the interaction of psoralen with UVA may contribute to CF formation in addition to superoxide from inflammatory cells, and this may contribute to photocarcinogenesis by PUVA therapy. Moreover, psoralen alone has been qualified as a well-known rodent carcinogen (Kevekordes et al. 2001). Psoralen causes a significant increase in the number of MN in human lymphocytes (Kevekordes et al. 2001). It has been argued that combination of psoralen and UVA light were not simply additive but synergistic (Yurkow and Laskin 1991).

In the case of PUVA, DNA repair time of human lymphoid cells has been found as 12 hours. (Kleinau et al. 1997). In this consideration, and because our patients have received maintenance PUVA therapy once or twice a week, this means that their DNA repair has been completed before the subsequent treatment. Therefore we assume that regular increase of MN observed by us during the different session of PUVA treatment cannot be attributable to incomplete DNA repair time course. In the light of our experiments it is plausible to admit that, once appeared, micronuclei do not disappear in the interval of the two consecutive PUVA treatments and rather they persists in the lymphocytes of the patients from a few days to a few weeks. Another investigation is needed to determine the precise time in which MN frequency reach to its initial level.

PUVA treatment is known to increase the risk of non-melanoma skin tumors (Tamada et al. 1997) such as squamous cell carcinoma (Nataraj et al. 1997; Stern et al. 1998; Takeda et al. 1998; Lindelöf et al. 1999), basal cell carcinoma (McKenna et al. 1996; Seidl et al. 2001) and in some extent of melanoma (Lindelöf 1999). However, reports on the haematopoietic cells malignancy after PUVA therapy are sporadic (Hansen 1979; Freeman and Warin 1985; Sheehan-Dare et al. 1989; Kwong et al. 1997; Takeoka et al. 2000) and some follow-up studies reported that compared to the control population their incidences are insignificant (Stern et al. 1997; Hannuksela-Svahn et al. 2000). Why is there more risk of skin malignancy after PUVA treatment compared to that of the haematopoietic cells like as leukaemia and malignant lymphoma?

PUVA is known not only as a carcinogen but also an immunosuppressor (Molin and Larkö 1997; Seidl et al. 2001). PUVA therapy-induced immunosuppression may play an important role in PUVA-related carcinogenesis (Weinstock et al. 1995). An increased frequency of cutaneous neoplasms in depigmented areas is a potential hazard, as the photoprotective effect of melanin in these sites is lacking (Buckley and Rogers 1996). PUVA induced immunosuppression may play an important role in PUVA related carcinogenesis by affecting the extent and pathogenicity of sunlamps/sunbeds (Armstrong and Kricker 1995), chronic sun exposure (Seidl et al. 2001) and human papillomavirus infections (Weinstock et al. 1995). It is plausible to admit that the synergistic effect of UVA/psoralen, by consequence immunosuppression and cellular damages during PUVA treatment are higher in UVA irradiated sites than that of the other parts of the body. As it was recommend by Lindelöf et al. (1999), patients should be carefully selected for PUVA and rigorously followed up. To conclude, as the residual damage of the PUVA as MN in lymphocytes is apparent, therapists should be careful with its

use, particularly on the patients who may be more sensitive to carcinogenesis.

References

Ahmed, N.U., Ueda, M., Nikaido, O., Osawa, T. & Ichihashi, M. (1999) High levels of 8-hydroxy-2′-deoxyguanosine appear in normal human epidermis after a single dose of ultraviolet radiation. *Br. J. Dermatol.*, **140**, 226–231.

Andriadze, M.I., Pleskach, N.M., Mikhelson, V.M. & Zhestyanikov, V.D. (1987) Sister-chromatid exchanges in a special form of xeroderma pigmentosum (form II). *Mutat. Res.*, **180**, 89-92.

Armstrong, B.K. & Kricker, A. (1995) Skin cancer. *Dermatol. Clin.*, **13**, 583–594.

Averbeck, D., Dardalhon, M. & Magana-Schwencke, N. (1990) Repair of Furucoumarin-plus-UVA-induced damage and mutagenic consequences in eukaryotic cells. *J. Photochem. Photobiol.*, **6**, 221-236.

Baker, H. & Wilkinson, D.S. (1979) Photochemotherapy. In: *Textbook of Dermatology*, edited by A. Rook, D.S. Wilkinson & F.J.G. Ebling, Blackwell Sci. Publ., Oxford, pp. 2248-52.

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

Buckley, D.A. & Rogers, S. (1996) Multiple keratoses and squamous carcinoma after PUVA treatment of vitiligo. *Clin. Exp. Dermatol.*, **21**, 43–45.

Bush, C. & McMillan, T.J. (1993) Micronucleus formation in human tumour cells: lack of correlation with radiosensivity. *Br. J. Cancer*, **67**, 102–106.

Catena, C., Conti, D., Villani, P., Nastasi, R., Archilei, R. & Righi, E. (1994) Micronuclei and 3AB index in human and canine lymphocytes after in vitro X-irradiation. *Mutat. Res.*, **312**, 1-8.

Cooke, M.S., Evans, M.D., Burd, R.M., Patel, K., Barnard, A., Lunec, C. & Hutchinson, P.E. (2001) Induction and excretion of ultraviolet-induced 8-oxo-2′-deoxyguanosine and thymine dimers in vivo: implications for PUVA. *J. Invest. Dermatol.*, **116**, 281-285.

Coven, T.R., Walters, I.B., Cardinale, I. & Krueger, J.G. (1999) PUVA-induced lymphocyte apoptosis: mechanism of action in psoriasis. *Photodermatol. Photoimmunol. Photomed.*, **15**, 22-27.

Darroudi, F. & Natarajan, A.T. (1987) Cytological characterization of Chinese hamster ovary x-ray-sensitive mutant cells, xrs 5 and xrs 6. II. Induction of sister-chromatid exchanges and chromosomal aberrations by x-rays and UV-irradiation and their modulation by inhibitors of poly (ADP-ribose) synthetase and alpha-polymerase. *Mutat. Res.*, **177**, 149-160.

Enomoto, D.N., Schellekens, P.T., Yong, S.L., ten Berge, I.J., Mekkes, J.R. & Bos, J.D. (1997) Extracorporeal photochemotherapy (photopheresis) induces apoptosis in lymphocytes: a possible mechanism of action of PUVA therapy. *Photochem. Photobiol.*, **65**, 177-180.

Falck, G., Catalan, J. & Norppa, H. (1997) Influence of culture time on the frequency and contents of human lymphocyte micronuclei with and without cytochalasin B. *Mutat. Res.*, **392**, 71-79.

Fenech, M. (1997) The advantages and disadvantages of the cytokinesis -block micronucleus method. *Mutat. Res.*, **392**, 11-18.

Filipe, P., Emerit, I., Alaoui, Y.A., Levy A., Cernjavski, L., Freitas, J. & de Castro, J.L. (1997) Oxyradical-mediated clastogenic plasma factors in psoriasis: Increase in clastogenic activity after PUVA. *Photochem. Photobiol.*, **66**, 497-501.

Forni, A. (1994) Comparison of chromosome aberrations and micronuclei in testing genotoxicity in humans. *Toxicol. Lett.*, **72**, 185-190.

Freeman, K. & Warin, A.P. (1985) Acute myelomonocytic leukaemia developing in a patient with psoriasis treated with oral 8-methoxypsoralen and longwave ultraviolet light. *Clin. Exp. Dermatol.*, **10**, 144-146.

Gasparro, F.P., Felli, A. & Schmitt, I.M. (1997) Psoralen photobiology: The relationship between DNA damage, chromatin structure, transcription and immunogenic effects. *Recent Results Cancer Res.*, **143**, 101-127.

Gocke, E., Muller, L., Guzzie, P.J., Brendler-Schwaab, S., Bulera, S., Chignell, C.F., Henderson, L.M., Jacobs, A., Murli, H., Snyder, R.D. & Tanaka, N. (2000) Consideration on photochemical genotoxicity: Repor of the international workshop on genotoxicity test procedures working group. *Environ.*

Mol. Mutagen., **35**, 173-184.

Hamurcu, Z., Donmez, H., Saraymen, R. & Demirtas, H. (2001) Micronucleus frequencies in workers exposed to lead, zinc and cadmium. *Biol. Trace Elem. Res.*, **83**, 97-102.

Hannuksela-Svahn, A., Pukkala, E., Laara, E., Poikolainen, K. & Karvonen, J. (2000) Psoriasis, its treatment, and cancer in a cohort of Finnish patients. *J. Invest. Dermatol.*, **114**, 587-590.

Hansen, N.E. (1979) Development of acute myeloid leukaemia in a patient with psoriasis treated with oral 8-methoxypsoralen and longwave ultraviolet light. *Scand. J. Haematol.*, **22**, 57-60.

Hashimoto, K. & Kumakiri, M. (1979) Colloid-amyloid bodies in PUVA-treated human psoriatic patients. *J. Invest. Dermatol.*, **72**, 70-80.

He, J., Chen, W., Jin, L.& Jin, H. (2000) Comet assay and cytokinesis-blocked micronucleus test for monitoring the genotoxic effect of X ray radiation in human. *Chin. Med. J.*, **113**, 911-914.

Heddle, J.A. (1973) A rapid in vivo test for chromosome damage. *Mutat. Res.*, **18**, 187-190.

Johnson, R., Staiano-Coico, L., Austin, L., Cardinale, I., Nabeya-Tsukifuji, R. & Krueger, J.G. (1996) PUVA treatment selectively induces a cell cycle block and subsequent apoptosis in human T-lymphocytes. *Photochem. Photobiol.*, **63**, 566-571.

Kevekordes, S., Spielberger, J., Burghaus, C.M., Birkenkamp, P., Zietz, B., Paufler, P., Diez, M., Bolten, C. & Dunkelberg, H. (2001) Micronucleus formation in human lymphocytes and in the metabolically competent human hepatoma cell line Hep-G2: Results with 15 naturally occurring substances. *Anticancer Res.*, **21**(1A), 461-469.

Kirsch-Volders, M., Sofuni, T., Aardema, M., Albertini, S., Eastmond, D., Fenech, M., Ishidate, M., Jr., Lorge, E., Norppa, H., Surrales, J., Von der Hude, W. & Wakata, A. (2000) Report from the in vitro micronucleus assay working group. *Environ. Mol. Mutagen.*, **35**, 167-172.

Kleinau, O., Böhm, F. & Lanto, B. (1997) Different DNA repair time courses in human lymphoid cells after UVA, UVA1, UVB and PUVA in vitro. *J. Photochem. Photobiol. B.*, **41**, 103-108.

Knobler, R.M., Hönigsmann, H. & Edelson, R.L. (1988) Psoralen phototherapies, In: *Psoralen DNA Photobiology*, edited by F.P. Gasparro, Vol. II, CRC Press, Boca Raton, FL, pp. 117-133.

Krishna, G. & Hayashi, M. (2000) In vivo rodent micronucleus assay: protocol, conduct and data interpretation. *Mutat. Res.*, **455**, 155-166.

Kwong, Y.L. Au, W.Y., Ng, M.H., Chan, L.C. & Au, T.S. (1997) Acute myeloid leukaemia following psoralen with ultraviolet A therapy: A fluorescence in situ hybridisation study. *Cancer Genet. Cytogenet.*, **99**, 11-13.

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

Laporte, M., Galand, P., Fokan, D., de Graef, C. & Heenen, M. (2000) Apoptosis in established and healing psoriasis. *Dermatology*, **200**, 314-316.

Liang, J.C., Hsu, T.C. & Henry, J.E. (1983) Cytogenetic assays for mitotic poisons. The grasshopper embryo system for volatile liquids. *Mutat. Res.*, **113**, 467-479.

Lindelöf, B. (1999) Risk of melanoma with psoralen/ultraviolet A therapy for psoriasis. Do the known risks now outweigh the benefits? *Drug. Saf.*, **20**, 289-297.

Lindelöf, B., Sigurgeirsson, B., Tegner, E., Larkö, O., Johannesson, A., Berne, B., Ljunggren, B., Andersson, T., Molin, L., Nylander-Lundqvist, E. & Emtestam, L. (1999) PUVA and cancer risk: the Swedish follow-up study. *Br. J. Dermatol.*, **141**, 108-112.

Liu, Z., Lu, Y., Lebwohl, M. & Wie, H. (1999) PUVA (8-methoxy-psoralen plus ultraviolet A) induces the formation of 8-hydroxy-2′-deoxyguanosine and DNA fragmentation in calf thymus DNA and human epidermoid carcinoma cells. *Free Radic. Biol. Med.*, **27**, 127-133.

McKenna, K.E., Patterson, C.C., Handley, J., McGinn, S. & Allen, G. (1996) Cutaneous neoplasia following PUVA therapy for psoriasis. *Br. J. Dermatol.*, **134**, 639-642.

Meunier, J.-R., Sarasin, A. & Marrot, L. (2002) Photogenotoxicity of mammalian cells: A review of different assays for in vitro testing.

Photochem. Photobiol., **75**, 437–447.

Molin, L. & Larkö, O. (1997) Cancer induction by immunosuppression in psoriasis after heavy PUVA treatment. *Acta Derm. Venereol.*, **77**, 402.

Mori, M., Kobayashi, H., Katsumura, Y. & Furihata, C. (2001) Induction of unscheduled DNA synthesis in hairless mouse epidermis by 8-methoxypsoralen plus ultraviolet A (PUVA). *J. Toxicol. Sci.*, **26**, 1–8.

Morita, A., Werfel, T., Stege, H., Ahrens, C., Karmann, K., Grewe, M., Grether-Beck, S., Ruzicka, T., Kapp, A., Klotz, L.O., Sies, H. & Krutmann, J. (1997) Evidence that singlet oxygen-induced human T helper cell apoptosis is the basic mechanism of ultraviolet-A radiation phototherapy. *J. Exp. Med.*, **186**, 1763–1768.

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

Möller, M., Stopper, H., Haring, M., Schleger, Y., Epe, B., Adam, W. & Saha-Möller, C.R. (1995) Genotoxicity induced by furocoumarin hydroperoxides in mammalian cells upon UVA irradiation. *Biochem. Biophys. Res. Commun.*, **216**, 693–701.

Nataraj, A.J., Wolf, P., Cerroni, L. & Anantatsaswamy, H.N. (1997) p53 mutation in squamous cell carcinomas from psoriasis patients treated with psoralen+UVA (PUVA). *J. Invest. Dermatol.*, **109**, 238–243.

Nevala, H., Karenko, L., Vakeva, L. & Ranki, A. (2001) Proapoptotic and antiapoptotic markers in cutaneous T-cell lymphoma skin infiltrates and lymphomatoid papulosis. *Br. J. Dermatol.*, **145**, 928–937.

Odagiri, Y., Uchida, H. & Shibazaki, S. (1997) Interindividual variation in cytogenetic response to X-ray and colchicine measured with the cytokinesis-block micronucleus assay. *Mutat. Res.*, **381**, 1–13.

Otrin, V.R., McLenigan, M., Takao, M., Levine, A.S. & Protic, M. (1997) Translocation of a UV-damaged DNA binding protein into a tight association with chromatin after treatment of mammalian cells with UV light. *J. Cell Sci.*, **110**, 1159–1168.

Parrish, J.A., Stern, R.S., Pathak, M.A. & Fitzpatrick, O. (1982) Photochomotherapy of skin diseases, In: *The Science of Photomedicine*, edited by J.D. Regan & J.A. Parrish, Plenum, New York, pp. 595–624.

Pfeifer, G.P. (1997) Formation and processing of UV photoproducts: Effects of DNA sequence and chromatin environment. *Photochem. Photobiol.*, **65**, 270–283.

Pinero, J., Lopez Baena, M., Ortiz, T. & Cortes, F. (1996) Sister chromatid exchange induced by DNA topoisomerases poisons in late replicating heterochromatin: influence of inhibition of replication and transcription. *Mutat. Res.*, **354**, 195–201.

Rosin, M.P. (1992) The use of the micronucleus test on exfoliated cells to identify anti-clastogenic action in humans: a biological marker for the efficacy of chemopreventive agents. *Mutat. Res.*, **267**, 265–276.

Sardas, S., Karahalil, B., Karakaya, A.E. & Sasmaz, R. (1994) Mutagenic risk in psoriatic patients before and after 8-methoxypsoralen and long-wave ultraviolet radiation. *Mutat. Res.*, **312**, 79–83.

Schmid, W. (1975) The micronucleus test. *Mutat. Res.*, **31**, 9–15.

Schwartz, J.L. & Weichselbaum, R.R. (1985) Antipain-mediated suppression of sister chromatid exchanges induced by an inhibitor of poly (ADP-ribose) polymerase. *Environ. Mutagen.*, **7**, 703–709.

Seidl, H., Kreimer-Erlacher, H., Back, B., Soyer, H.P., Höfler, G., Kerl, H. & Wolf, P. (2001) Ultraviolet exposure as the main initiator of p53 mutations in basal cell carcinomas from psoralen and ultraviolet A-treated patients with psoriasis. *J. Invest. Dermatol.*, **117**, 365–370.

Sheehan-Dare, R.A., Cotterill, J.A. & Barnard, D.L. (1989) Transformation of myelodysplasia to acute myeloid leukaemia during psoralen photochemotherapy (PUVA) treatment of psoriasis. *Acta Derm. Venereol.*, **69**, 262–264.

Snyder, R.D. & Cooper, C.S. (1999) Photogenotoxicity of fluoroquinolones in Chinese hamster V79 cells: dependency on active topoisomerase II. *Photochem. Photobiol.*, **69**, 288–293.

Sonoda, E., Sasaki, M.S., Morrison, C., Yamaguchi-Iwai, Y., Takata, M. & Takeda, S. (1999) Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells. *Mol. Cell Biol.*, **19**, 5166–5169.

Stern, R.S., Vakeva, L.H. & the PUVA follow-up study. (1997) Noncutaneous malignant tumors in the PUVA follow-up study: 1975-1996. *J. Invest. Dermatol.*, **108**, 897-900.

Stern, R.S., Liebman, E.J. & Vakeva, L. (1998) Oral psoralen and ultraviolet-A light (PUVA) treatment of psoriasis and persistent risk of nonmelanoma skin cancer. PUVA follow-up study. *J. Natl. Cancer Inst.*, **90**, 1278-1284.

Takeda, H., Mitsuhashi, Y. & Kondo, S. (1998) Multible squamous cell carcinoma in situ in vitiligo lesions after long-term PUVA therapy. *J. Am. Acad. Dermatol.*, **38**, 268-270.

Takemori, N., Hirai, K. Onodera, R., Saito, N., Yokota, K., Kinoushi, M. & Lizuka, H. (1995) Satisfactory remission achieved by PUVA therapy in a case of crisis-type adult T-cell leukaemia/lymphoma with generalized cutaneous leukaemic cell infiltration. *Br.J. Dermatol.*, **133**, 955-960.

Takeoka, Y., Yamane, T., Koh, K.R., Ohta, K., Nakamae, H., Aoyama, Y., Hirose, A., Fujino, Y., Inoue, T., Hino, M. & Tatsumi, N. (2000) Mycosis fungoides terminating in acute myelocytic leukaemia. *Rinsho Ketsueki*, **41**, 755-760.

Tamada, Y., Arisawa, S., Ikeya, T. & Hara, K. (1997) Non-melanoma skin tumors occurring on a Japanese psoriatic patient treated with long-term photochemotherapy. *J. Dermatol.*, **24**, 552-553.

Thust, R., Schacke, M. & Wutzler, P. (1996) Cytogenetic genotoxicity of antiherpes virostatics in Chinese hamster V79-E cells. I. Purine nucleoside analogues. *Antiviral. Res.*, **31**, 105-113.

Tice, R.R., Agurell, E., Anderson D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.-C. & Sasaki, Y.F. (2000) Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. *Environ. Mol. Mutagen.*, **35**, 206-221.

Weinstock, M.A., Coulter, S., Bates, J., Bogaars, H.A., Larson, P.L. & Burmer, G.C. (1995) Human papillomavirus and widespread cutaneous carcinoma after PUVA photochemotherapy. *Arch. Dermatol.*, **131**, 701-704.

Wickenhauser, C., Borchmann, P., Diehl, V. & Scharffetter-Kochanek, K. (1999) Development of IGG lambda multible myeloma in a patient with cutaneous CD30+ anaplastic T-cell lymphoma. *Leuk. Lymphoma*, **35**, 201-206.

Yoo, E.K., Rook, A.H., Elenitsas, R., Gasparro, F.P. & Vowels, B.R. (1996) Apoptosis induction of ultraviolet light A and photochemotherapy in cutaneous T-cell Lymphoma: relevance to mechanism of therapeutic action. *J. Invest. Dermatol.*, **107**, 235-242.

Yurkow, E.J. & Laskin, J.D. (1991) Mechanism of action of psoralens: Isobologram analysis reveals that ultraviolet light potentiation of psoralen action is not additive but synergistic. *Cancer Chemother. Pharmacol.*, **27**, 315-319.