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THE BINDING OF 8-METHOXYPSORALEN TO NUCLEAR DNA OF UVA IRRADIATED HUMAN FIBROBLASTS IN VITRO

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Abstract. Human fibroblasts were exposed to tritium-labelled 8-methoxypsoralen (8-MOP) and longwave ultraviolet (UVA) light in vitro. The proportions of photochemically bound 8-MOP in the nucleus and cytoplasm were studied by autoradiography. The results show that UVA-irradiation of the cells in the presence of 8-MOP gives rise to a significant dose-dependent binding of 8-MOP in the cell nucleus.

Key words: Psoriasis; Psoralen; Ultraviolet light; Deoxyribonucleic acid (DNA); Human fibroblasts; Autoradiography

Treatment of psoriasis with oral psoralen followed by irradiation of the skin with UVA light (PUVA treatment) gives a good clinical effect (5, 9, 12). There is increasing concern, however, regarding the possible mutagenic effect of PUVA treatment on somatic cells (10). UVA irradiation of purified DNA in the presence of 8-methoxypsoralen (8-MOP) produces various types of monoadducts and cross-links (2, 3). Other types of photochemical reaction are also possible with 8-MOP (7). Interstrand cross-links (binding together of the two DNA strands, probably often leading to inhibition of mitosis) are formed in guinea pig cells in vivo after PUVA treatment (4). PUVA exposure of yeast cells yield respiration deficient mutants (11); i.e. the mitochondrial mutations have bereft the yeast cells of their respiratory capacity. Exposure of human cells in vitro to PUVA gives rise to chromosome aberrations (9) and induces DNA repair synthesis (1).

Thus, PUVA treatment seems to damage chromosomal DNA in the cell nucleus as well as mitochondrial DNA in the cytoplasm. If the clinical effect is mediated mainly via respiratory derangement (mitochondrial damage) and not by damaging the gene material of the nucleus, the risk of impeding the growth control of the cell (i.e. induce tumour formation) is certainly very small. If, on the other hand, PUVA treatment in clinical doses can be shown to cause damage to nuclear DNA, additional studies would be warranted to assess more closely the risk of unwanted side-effects. Such studies should also include other, conventional treatments of psoriasis for comparison (10).

In this work we demonstrate that clinically used doses of PUVA, when applied to human fibroblasts in vitro, yield a dose-related increase of 8-MOP in the cell nucleus.

MATERIAL AND METHODS

Fibroblasts were obtained from skin biopsy material from a healthy human subject. The cells were grown as monolayers on coverslips in Leighton tubes containing Eagle's Minimal Essential Medium (MEM), supplemented with 20% fetal calf serum, 0.3 mg L-glutamine/ml, 80 μg streptomycin/ml and 80 IE benzylpenicillin/ml. Before the cells covered the entire surface, the coverslips were gently removed from the tubes and transferred to Petri dishes containing 7.5 ml Eagle's MEM. Tritium-labelled 8-MOP (1.67 Ci/mM, New England Nuclear) dissolved in ethanol (0.65 mg/5 ml) was added in concentrations indicated below. The cells were incubated for 30 min and then irradiated for 20 min in UVA-light from a Black-ray B-100A lamp (Ultra-Violet Products Inc.). Non-irradiated cells served as controls. The coverslips were then washed three times in phosphate-buffered saline and kept in fixative (methanol/acetic acid 3 : 1) for 30 min. From the addition of 8-MOP until fixation only red light was permitted. The cells were then washed and dehydrated in step-wise increasing concentrations of ethanol. After drying in air the cover slips were mounted on object slides, and covered with autoradiographic film (Kodak AF 10). After 21 weeks of exposure the slides were developed and the cells stained in Giemsa (5% in Sörensen buffer solution, pH 6.8) for 4–8 minutes. The slides were coded and microscopic analysis was done blind. A grid-patterned ocular was used and the following criteria for scoring

Table I. *Autoradiographic analysis of PUVA treated fibroblasts*

Experiment	8-MOP dose (μg/ml)	UVA dose (J/cm²)	No. of cells analysed	Mean no. of grains per square of		
				Background	Cytoplasm	Nucleus
A	8.1	None	15	0.3	5.6	6.1
B	3.4	6.0	14	0.5	3.0	6.5
C	8.1	6.0	10	0.4	3.3	11.9
D	8.1	6.0	11	0.4	2.7	10.4

grains were adopted; the cell to be analysed should be completely separate from other cells; the boundary between nucleus and cytoplasm should be clearly visible; an equal number of graticule squares in the nucleus, cytoplasm and background close to the cell should be scorable, the number being determined by which of the areas is the smaller—the nucleus or the cytoplasm.

RESULTS AND DISCUSSION

The experiments and results are summarized in Table I. Control cells (A) receiving the highest 8-MOP dose but no UVA light displayed an almost equal grain density over the nucleus and cytoplasm. This labelling was probably due to intracellularly fixed 8-MOP, which is not photochemically bound, yet cannot be completely removed by the washing procedure. Cells which were exposed to both 8-MOP and UVA light (B, C and D) displayed a higher grain density over the nucleus than over the cytoplasm, which indicates that UVA irradiation makes 8-MOP bind preferentially to intranuclear structures. There was a clear dose effect, as seen from the increasing nuclear grain density with increasing 8-MOP dose (B versus the identical experiments C and D).

Table II. *Nucleus/cytoplasm quotients of mean grain number per ocular square of each cell*

Experimental details as in Table I

Cell no.	Experiment			
	A	B	C	D
1	0.6	1.5	2.0	2.7
2	0.6	1.6	2.3	2.9
3	0.9	1.7	3.0	3.0
4	1.0	2.0	3.2	3.5
5	1.0	2.0	3.7	4.4
6	1.0	2.1	3.7	4.6
7	1.1	2.2	4.8	5.5
8	1.1	3.2	5.8	6.3
9	1.1	3.2	6.5	7.0
10	1.2	3.2	12.8	7.5
11	1.3	3.3		7.8
12	1.4	3.5		
13	1.6	5.1		
14	2.1	23.0		
15	3.6			

The large number of grains over the cytoplasm of unirradiated cells (A) and the drop in cytoplasmic grain count when the cells are exposed to UVA (B, C and D) may seem confusing. In B the low cytoplasmic grain count can be explained by the fact that the 8-MOP dose is 2.5 times lower than in A, and consequently the nuclear grain density in B can be regarded as a substantial relative increase in binding of 8-MOP compared with A. In C and in D the dose of 8-MOP is identical with A. The lower cytoplasmic counts in these experiments are probably due to a competitive mechanism arising when 8-MOP is fixed in the nucleus at the expense of 8-MOP available in the cytoplasm.

Statistical analyses of the results were based on calculation of the quotient between grain density in the nucleus and in the cytoplasm of each cell after subtraction of the surrounding background labelling (Table II). The quotients obtained in each of the experiments were compared with those of the other experiments, by the Mann-Whitney rank test for samples of unequal size (8). The analysis showed that the nucleus/cytoplasm quotients of experiments B, C and D were significantly higher ($P<0.001$, one-tailed test) than those of experiment A, and the quotients of C and D were significantly higher than those of experiment B ($P<0.05$ and $P<0.01$ respectively, one-tailed test). No statistical difference was found between C and D.

Thus our results demonstrate that UVA irradiation of human fibroblasts in the presence of 8-MOP gives rise to a significant, dose-dependent binding of 8-MOP in the cell nucleus, probably mainly to nuclear DNA. No information about the possibility that an effect on mitochondrial genes may be of importance in the PUVA treatment can be derived from the present study, since mitochondrial DNA

amounts to only about one-thousandth part of that in the nucleus. Thus, even if there were a considerable increase in 8-MOP bound to mitochondrial DNA, this would not significantly alter the grain ratios studied.

The present demonstration of UVA mediated binding of 8-MOP in the cell nucleus is in agreement with the results of Pathak et al. (6) using cell fractionation procedures. It also correlates well with our previous findings of chromosome aberrations (9) and our preliminary observation of sister chromatid exchanges caused by PUVA treatment of human cells in vitro. We therefore feel that, parallel with the extension of clinical PUVA therapy, additional studies on the genetic effects of PUVA treatment in somatic cells should be carried out.

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