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Increased proliferation of phytohaemagglutinin (PHA)-stimulated human leucocytes after 8-methoxypsoralen treatment

A. GAWRON, G. GÓRSKI*, K. GŁOWNIAK**, *Department of Cell Biology, M. Curie-Skłodowska University, Lublin, Poland, *Department of Medical Genetic, Medical Academy, Lublin, Poland and **Department of Pharmacognosy, Medical Academy, Lublin, Poland*

Abstract—The effect of 8-methoxypsoralen on human blood mononuclear cells cultured in darkness has been examined. 8-Methoxypsoralen (1 μg mL^{-1}, 4·6 μM) added to (phytohaemagglutinin-stimulated cells induced a two-fold increase in the number of mitoses. The number of blastic cells also increased. There was no effect on the formation of micronuclei and there was no chromosomal breakage.

8-Methoxypsoralen (8-MOP) is efficacious when combined with ultraviolet A light (UVA) in the management of some cutaneous disorders such as psoriasis and vitiligo (Parrish et al 1974) and in the management of the early skin phase of cutaneous T-cell lymphoma (Edelson et al 1983).

8-MOP given orally may affect various cells, including blood cells. Studies on its effect on lymphocytes showed that after UVA activation it has a strong inhibitory effect on the proliferation of phytohaemagglutinin (PHA)-stimulated lymphocytes. Moreover, 8-MOP simultaneously induced an increase in sister chromatid exchange (SCE) and numerous chromosome aberrations (Latt & Loveday 1978; Berger et al 1985).

The mechanism of action of 8-MOP on lymphocyte proliferation without photoactivation, is not understood. Some reports indicate no effect (Scherer et al 1977; Gast et al 1985), others describe a negative effect on viability and proliferation as well as mutagenic action (Lischka & Decker 1978; Abel & Schimmer 1981).

We now report the results of the action of 8-MOP at clinical concentration on PHA-stimulated leucocytes.

Materials and methods

8-Methoxypsoralen was isolated as described by Głowniak et al (1986). It was used chromatographically pure in solution in dimethyl sulphoxide (Merck) for these studies.

Human leucocytes derived by sedimentation from peripheral blood of six healthy individuals were grown in culture to give a concentration of 1×10^6 mL^{-1}. The culture medium consisted of 80% Eagle's MEM 1959 (WSIS, Lublin), 20% inactivated calf serum (WSIS, Lublin) supplemented with 2 mM L-glutamine (Reanal, Hungary), 25 mM HEPES buffer pH 7·3 (Ubichem, USA), penicillin 100 iu mL^{-1}, streptomycin 100 μg mL^{-1}, mycostatin 25 iu mL^{-1}. Cultured cells were stimulated by PHA HA-15 (Wellcome, UK) (20 μg mL^{-1}) and incubated at 37°C.

Two cultures were established for each donor. Cultures were incubated in darkness. After 72 h incubation, 8-MOP in a final concentration of 1 μg mL^{-1} (4·6 μM) was added to one sample with the second as control. Twenty four hours later the cultures were harvested using 0·075 M KCl and fixed by Carnoy fixative solution. Two h before harvest, Colcemid (CIBA) was added to give a final concentration of 0·1 μg mL^{-1}. The air dried slides were stained with 0·04% Giemsa (BDH) solution for 10–15 min. Each slide was examined by two observers. About 500 cells were scored for the determination of the mitotic and blastic index and about 1000 cells were tested for the presence of micronuclei. Fifty metaphases were analysed to determine chromosomal aberrations. The results of six experiments were analysed statistically by use of the χ^2 test at $P < 0.05$ for significance.

Correspondence to: A. Gawron Department of Cell Biology, M. Curie-Skłodowska University, ul. Akademicka 19, PL-20-033 Lublin, Poland.

Results

The effect of 8-MOP on the proliferation of leucocytes was evaluated by mitotic and blastic index calculations. The results are shown in Tables 1 and 2.

Table. 1. The effect of 8-methoxypsoralen (8-MOP, 1 μg mL^{-1}) on mitotic index of PHA-stimulated leucocytes.

Experiment	No. of cells	No. of mitoses	Mitotic index ‰
1. Control	460	10	21·7
8-MOP	448	19	42·4***
2. Control	442	7	15·8
8-MOP	431	18	41·8**
3. Control	461	13	30·0
8-MOP	441	14	31·7
4. Control	525	3	5·7
8-MOP	545	10	18·3**
5. Control	570	20	35·1
8-MOP	550	48	87·3***
6. Control	520	12	23·1
8-MOP	510	20	39·2*
Mean			
Control	496	11	21·9
8-MOP	487	22	43·5***

Each value represents the average of two determinations.
Significant differences from control *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. The effect of 8-methoxypsoralen (8-MOP, 1 μg mL^{-1}) on blastic index of PHA-stimulated leucocytes.

Experiment	No. of cells	No. of blastic cells	Blastic index ‰
1. Control	460	192	417·3
8-MOP	448	220	491·0*
2. Control	442	189	427·6
8-MOP	431	242	561·5***
3. Control	461	111	241·0
8-MOP	441	195	442·0***
4. Control	525	208	396·2
8-MOP	545	301	552·3***
5. Control	570	260	456·1
8-MOP	550	315	572·7**
6. Control	520	251	482·7
8-MOP	510	272	533·3*
Mean			
Control	496	202	403·5
8-MOP	487	257	525·5***

Each value represents the average of two determinations.
Significant difference from control: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

In all cultures of PHA-stimulated cells treated with 8-MOP, the mitotic index was higher compared with controls. In most cultures studied the number of mitotic cells increased two fold, and in one instance three fold compared with control cultures. Only in one experiment was the mitotic index close to that of the control.

The blastic index in cultures with 8-MOP was higher than in controls. However, its increase was not as great as that of the mitotic index, although the differences were statistically significant in five out of six cases.

The micronucleus test and microscopic chromosome studies were used to assess possible chromosome damage. Microscopical studies of about 50 metaphases showed no chromosomal aberrations in the control or 8-MOP-treated cells. Although the number of cells with micronuclei formation in cultures treated with 8-MOP was higher than in the control in four experiments, the observed differences were not statistically significant (means were: control, 993 range 920–1140; 8-MOP, 995 range 862–1100: % cells with micronuclei 0·98 and 1·22, respectively).

Discussion

The effect of 8-MOP on PHA-stimulated leucocytes cultivated in darkness has been analysed. The concentration of 8-MOP in our experiments was comparable to the peak concentration in the serum of patients treated orally with 40 mg 8-MOP (Busch et al 1978; Stolk & Siddiqui 1988). We have found that 8-MOP, 1 μg mL^{-1}, increases leucocyte proliferation but only with cells previously stimulated with PHA. 8-MOP had no effect on non-stimulated cells (unpublished data). The increase in cell proliferation is in contradiction to the results of other authors claiming no effect of 8-MOP at therapeutic concentrations (Scherer et al 1977; Gast et al 1985).

The differences in our data from those of others may be the result of differences in 8-MOP purity or in culture conditions.

Abel & Schimmer (1981) have shown that 8-MOP and 8-isoamylenoxypsoralen (in concentrations twice those used by us) successively inhibited and accelerated lymphocyte proliferation whereas 5′-methylangelicin appears to accelerate rather than to inhibit the proliferation. Thornes et al (1983) noticed that when coumarin at a dose of 100 mg daily, was administered to patients with cancer or brucelosis, it first increased the number of helper T-lymphocytes. Our results suggest that an ability to increase PHA-stimulated leucocyte proliferation may be a common feature of the coumarin group of compounds.

Our observation that 8-MOP at therapeutic concentrations had no effect on chromosome damage in-vitro is in agreement with studies by other authors (Brogger et al 1978; Billardon & Moustacchi 1986). In addition, we have demonstrated that with our culture condition, 8-MOP in darkness induced neither chromosomal breakage nor micronucleus formation.

References

Abel, G., Schimmer, O. (1981) Effect on chromosomes by coumarin derivatives in the dark. Planta Medica. 42: 333–343

Berger, C. L., Cantor, Ch., Welsch, J. et al (1985) Comparison of synthetic psoralen derivatives and 8-MOP in the inhibition of lymphocyte proliferation. Ann. N.Y. Acad. Sci. 453: 80–90

Billardon, B., Moustacchi, E. (1986) Comparison of the sensitivity of fanconi anemia and normal fibroblasts to the induction of SCE by photoaddition of mono and bi-functional psoralens. Mutat. Res. 174: 241–246

Brogger, A., Waksvik, H., Thune, P. (1978) Psoralen, UVA treatment and chromosomes. Arch. Derm. Res. 261: 287–294

Busch, U., Schmid, J., Koss, F. W., Zipp, H., Zimmer, A. (1978) Pharmacokinetics and metabolite-pattern of 8-methoxypsoralen in man following oral administration as compared to the pharmacokinetics in rat and dog. Ibid. 262: 255–265

Edelson, R., Berger, C. L., Gasparro, F., Lee, K., Taylor, J. (1983) Treatment of leucemic cutaneous T-cell lymphoma with extracorporeally photoactivated 8-methoxypsoralen. Clin. Res. 31: 467 A

Gast, W., Walter, Th., Rytter, M., Barth. J., Haustein, U. F. (1985) Contribution to the effects of linear and angular psolarens on human lymphocytes. Vestn. Dermatol. Venerol. 8: 17–20

Głowniak, K., Wawrzynowicz, T. Soczewiński, E. (1986) Isolation of coumarins from the fruits of *pastinaca sativa* L. by column chromatography and modified thin-layer chromatography. Akademiai Kiado Budapest Chromatography 85: 251–261

Latt, S. A., Loveday, K. S. (1978) Characterization of sister chromatid exchange induction by 8-MOP plus near UV-light. Cytogenet. Cell Genet. 21: 184–200

Lischka, G., Decker, E. (1978) Dark effect of 8-MOP on lymphocytes. Arch. Dermatol. Res. 261: 267–272

Parrish, J. A., Fitzpatrick, T. B., Tannenbaum, L., Pathak, M. A. (1974) Photochemotherapy of psoriasis with oral methoxalen and longwave ultraviolet light. New. Engl. J. Med. 291: 1207–1211

Scherer, R., Kern, B., Braun-Falco, O. (1977). The human peripheral lymphocyte; a model system for studying the combined effect of psoralen plus black light. Klin. Wschr. 55: 137–140

Stolk, L. M. L., Siddiqui, A. H. (1988) Biopharmaceutics, pharmacokinetics and pharmacology of psoralens. Gen. Pharmacol. 19: 649–653

Thornes, R. D., Lynch, G., Sheehan, M. V. (1983) Coumarin and cimetidine in malignant melanoma. Ir. Med. J. 76: 53