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Effect of 8-methoxypsoralen in the dark on proliferation of stimulated lymphocytes

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Abstract—The effect of 8-methoxypsoralen (8-MOP) in the dark on proliferation of phytohaemagglutinin-stimulated human lymphocytes has been examined, using total blood leucocytes, the lymphocyte fraction and the T-lymphocyte subpopulation. 8-MOP (1 μg mL^{-1}) increased [^{3}H]TdR incorporation and caused a rise in the blastic and mitotic index in all experimental cultures. The lymphocyte proliferation in these experiments was effected by a comitogenic effect of 8-MOP on T-lymphocytes.

Orally administered 8-methoxypsoralen (8-MOP) together with UVA (PUVA) is efficacious in the management of psoriasis (Parrish et al 1974), vitiligo (Parrish et al 1976), mycosis fungoides (Roenigk 1977), as well as the early skin phase of cutaneous T-lymphoma (Edelson et al 1983). However, PUVA therapy has been reported to induce immunological abnormalities. These abnormalities include inhibited hypersensitivity reactions in skin (Kripke et al 1983), decreased leucocyte thymidine incorporation (Kraemer & Weinstein 1977; Kraemer et al 1981), diminished leucocyte responsiveness to phytohaemagglutinin (PHA) (Scherer et al 1977) and decreased circulating E-rosette forming T-lymphocytes (Haftek et al 1979).

The action of 8-MOP on lymphocyte proliferation without photoactivation is not understood. Some reports indicate no effect (Bohnert et al 1977; Gast et al 1985; Scherer 1977), while others describe a negative effect on viability and proliferation (Rytter et al 1982). We have reported an increased proliferation of PHA-stimulated human leucocytes in the presence of 8-MOP (Gawron et al 1990). We now report the results of the action of 8-MOP, in clinical doses, on stimulated leucocytes, lymphocytes and a T-lymphocyte subpopulation cultured in-vitro.

Materials and methods

The leucocytes, lymphocytes and T- and B-lymphocyte subpopulations were derived from peripheral blood from five healthy donors. Leucocytes were separated by sedimentation and suspended (1×10^6 cells mL^{-1}) in culture medium. Lymphocytes were isolated over Lymphoprep (Nycomed, Norway), washed three times in phosphate-buffered saline (PBS, WSiS—Serums and Vaccines Manufactory, Lublin, Poland) and counted. Half of the cells were suspended (1×10^6 mL^{-1}) in culture medium. The other half were used to prepare T- or B-lymphocyte fractions. These were separated by density sedimentation of spontaneous rosettes formed by T-lymphocytes and sheep red blood cells (SRBC) pre-treated with neuraminidase, Vibrio cholerae (Koch-Light), (Hirano et al 1977). The purity of T- and B-subpopulations was examined by surface staining with the monoclonal antibody Pan-T (Behring) and fluorescence microscopy. Thereafter, T- and B-lymphocytes were suspended in culture medium (1×10^6 mL^{-1}).

The culture medium consisted of 80% RPMI 1640 and 20% inactivated calf serum (WSiS, Lublin, Poland), supplemented with 2 mM L-glutamine (Reanal, Hungary), 25 mM HEPES buffer pH 7·3 (Ubichem, USA) penicillin 100 int. units mL^{-1}, streptomycin 100 μg mL^{-1} (Polfa, Poland) and mycostatin 25 int. units mL^{-1} (Calbiochem, USA). 8-Methoxypsoralen, isolated from angelica fruits, crystallized and chromatographically pure was received from Dr K. Głowniak (Dept. Pharmacognosy Medical Academy, Lublin, Poland).

Leucocytes, total lymphocytes and T-lymphocytes were stimulated with 1 μg mL^{-1} PHA-17 (Wellcome, UK). B-Lymphocytes were stimulated by SAC (*Staphylococcus aureus* Cowan) (Romagnani et al 1978).

Two cultures were established for leucocytes, total lymphocytes and T- and B-lymphocyte subpopulations. Cultures were incubated in the dark. After 72 h incubation, 8-MOP dissolved in dimethylsulphoxide (Merck) to a final concentration of 1 μg mL^{-1} (4·6 μM) was added to one sample while the second served as a control. Seven h later (17 h before harvest) [^{3}H]TdR (UVVVR, Czechoslovakia) was added to a final concentration of 2 μCi mL^{-1}. The cultures were harvested using 0·075 M KCl and fixed by five changes of Carnoy fixative solution. Two h before harvest, Colcemid (Ciba) was added to a final concentration of 0·1 μg mL^{-1}. Autoradiography was performed using microautoradiography strip film K106 (Orwo, Germany). After 14 days of exposure the slides were stained with 0·04% Giemsa (BDH, UK) solution for 5–10 min. One thousand cells were counted to determine the number of blastic cells, the number of blastic cells incorporating [^{3}H]TdR and radioactive mitoses.

The results were analysed by paired Student's *t*-test.

Results

Addition of 8-MOP (1 μg mL^{-1}) to cultures of PHA-stimulated cells caused an increase in the number of blastic cells, of cells incorporating [^{3}H]TdR, and of mitotic cells (Tables 1, 2, 3), in all experimental conditions studied. The lymphocytes fractionated into T+B cells incorporated most [^{3}H]TdR, total leucocytes incorporated less, and T-lymphocytes the least. The greatest differences in the number of blastic cells between cultures with 8-MOP and control were found in the cultures containing total blood lymphocytes.

Smaller differences were found in the number of mitoses between particular kinds of cultures. However, in all the cultures with 8-MOP the number of mitotic cells was almost three times greater than in the corresponding control.

Table 1. The effect of 8-methoxypsoralen (8-MOP, 1 μg mL^{-1}) on the number of blastic cells in three-day cultures.

Experimental system	Blastic cells (‰)	
	Control	8-MOP
Total blood leucocytes	586·6 ± 48·6	741·4 ± 82·4*
Total lymphocytes	447·2 ± 76·7	667·4 ± 105·7**
T-Lymphocytes	395·2 ± 77·0	518·0 ± 111·9*
B-Lymphocytes	551·4 ± 210·6	595·0 ± 153·5

Significant difference from control: * $P < 0·02$; ** $P < 0·001$.

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Table 2. The effect of 8-methoxypsoralen (8-MOP, 1 $\mu g\ mL^{-1}$) on the number of blastic cells incorporating [^{3}H]TdR in three-day cultures.

Experimental system	Cells incorporating[^{3}H]TdR (‰)	
	Control	8-MOP
Total blood leucocytes	378·0±118·8	572·0±161·9**
Total lymphocytes	234·4±81·9	381·6±67·1**
T-Lymphocytes	198·8±80·3	266·0±127·3*
B-Lymphocytes	12·2±10·6	9·6±8·0

Significant difference from control: * $P<0.05$; ** $P<0.001$.

Table 3. The effect of 8-methoxypsoralen (8-MOP, 1 $\mu g\ mL^{-1}$) on the number of mitotic cells in three-day cultures.

Experimental system	Mitotic cells (‰)	
	Control	8-MOP
Total blood leucocytes	7·2±2·9	23·2±10·9**
Total lymphocytes	3·4±1·5	10·1±4·1*
T-Lymphocytes	4·2±1·8	12·0±6·3*
B-Lymphocytes	no mitoses	

Significant difference from control: * $P<0.05$; ** $P<0.01$.

Discussion

8-MOP in therapeutic concentrations does not effect lymphocytes in the dark (Bohnert et al 1977; Gast et al 1985). A negative effect, i.e. a decrease in the proliferation appears only at high concentrations of 8-MOP (Scherer 1977; Rytter et al 1982). Our earlier studies have shown that 8-MOP (1 $\mu g\ mL^{-1}$) (i.e. at clinical values reached in the blood of treated subjects) can stimulate PHA-transformed leucocytes (Gawron et al 1990). In the present studies it was found that 8-MOP (1 $\mu g\ mL^{-1}$) enhanced the mitogenic effect of PHA on lymphocytes by acting on T-lymphocytes. However, the greatest increase in the incorporation of [^{3}H]TdR by cells was noted in cultures containing non-separated lymphocytes, which may be the result of the cell separation procedures disturbing lymphocyte viability. It could not be caused by the presence of B-lymphocytes; these are not stimulated by PHA or 8-MOP. In our research B-lymphocytes even if stimulated by SAC (a mitogen for these cells) showed many blastic forms but only slight incorporation of [^{3}H]TdR (Tables 1, 2) owing to the short time of culture. Therefore the differences in incorporation of [^{3}H]TdR were determined by the viability of the lymphocytes and not by their type. Nevertheless, the degree to which 8-MOP affected T-lymphocyte reactivity was different in different experiments, showing that 8-MOP heightened the effect of PHA on T-lymphocytes in-vitro.

In psoriatic patients subjected to PUVA treatment, an increase in T-lymphocytes has been observed (Haftek et al 1979). It is possible that this effect of 8-MOP on lymphocytes that are unaffected by UV, is the cause of the increased sensitivity of T-lymphocytes to mitogens and ultimately of an increased proliferation of lymphocytes in patients receiving psoralens.

Kripke et al (1983) reported an activation of suppressor lymphocytes in the spleen during PUVA. The mitogenic effect of psoralen in the dark was also noted by Arslan et al (1989). Comparing the effect of various coumarins on lymphocytes those authors found an unexpected 60–80% increase in [^{3}H]TdR incorporation in PHA stimulated lymphocytes in the presence of psoralen, and our present results are consistent with these findings. Although the mechanism of the effect of 8-MOP on lymphocytes is not known, it may involve the binding of 8-MOP with intracellular and extracellular receptors (Laskin et al 1985) or a change in cAMP (Albrightson et al 1985) under the influence of 8-MOP. Such an effect of 8-MOP may increase the reactivity of T-lymphocytes to mitogens and in consequence may cause an intensified proliferation of lymphocytes, as noted in our studies.

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