Dose-effects of 8-methoxypsoralen and long wave UV-light in 3T3 cells: evaluation of a phototoxic index[1]

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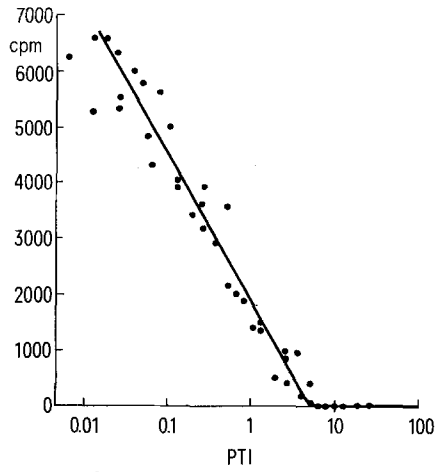
Summary. 3T3 cells were cultured until confluency and treated with various doses of 8-methoxypsoralen followed by long wave UV light irradiation. The inhibition of ^{3}H-thymidine incorporation was dose-dependent for both, psoralen and light. A phototoxic index (PTI) was established demonstrating that a constant correlation between psoralen and UVA light exists for the photoinactivation in living cells.

Psoralens are known to possess photosensitizing activity in bacteria, yeasts and animal cells after long wave UV irradiation (UVA). By UVA irradiation these substances bind as photo-mono-adducts to DNA or form interstrand cross-links in DNA[3-8]. Recently the use of 8-methoxypsoralen (8-MOP) together with potent UVA light sources has been found to be therapeutically effectful in various skin diseases. Although the mode of action in skin is not fully understood, the combined treatment is supposed to inhibit the proliferation of cutaneous cells.

In vivo the effectful dosage of 8-MOP and UVA has been found empirically[9,10] and appears to be considerably lower than the concentrations employed for in vitro experiments. Since biological inactivation after sensitization with psoralen plus UVA light appears to be dose-dependent, it is of interest to know the inactivating dosages of 8-MOP and UVA light, and the correlation of both.

Materials and methods. 3T3 cells (Swiss albino) were obtained from LS, Linbro culture dishes (FB-16-24-TC) from Flow Laboratories, 8-methoxypsoralen (8-MOP) from Basotherm, Earle's Balanced Salt Solution (EBSS), McCoy's 5A medium, fetal calf serum (10%) and penicillin/streptomycin (10,000 IU/10,000 µg/ml) from Gibco Bio-Cult. [methyl] ^{3}H-Thymidine was purchased from the Radiochemical Centre Amersham. 3T3 cells were seeded in Linbro culture dishes containing 1 ml McCoy's 5A medium supplemented with 10% fetal calf serum and 100 IU/ml penicillin and 100 µg/ml streptomycin. In humidified atmosphere containing 5% CO_2, the cells were allowed to grow to confluency at 37 °C. The growth medium was changed every 2 days. Subcultures were obtained every week by harvesting the cells by mild trypsinization (0.25% trypsin in EBSS) and re-seeding. 8-MOP (10^{-2}-10 µg/ml, dissolved in DMSO) was added at confluency together with fresh medium. The final concentration of DMSO in the culture medium was lower than 0.1%. The cell cultures were kept for 1 h in the dark, followed by irradiation with UVA (PUVA 4000, Waldmann, Germany; output 6.5 mW/cm^2) at different dosages (0.65, 1.3, 1.95, 2.6 J/cm^2). Absorption of UVA radiation by medium and the cover of the culture dishes was taken into account. The 8-MOP containing medium was removed and replaced by fresh medium. 24 h after exposure to UVA irradiation, 2 µCi/ml of ^{3}H-thymidine was added to the culture medium for 2 h. After several washes with EBSS and mild trypsinization, the cells were harvested (Titertek, Flow Laboratories) on filter plates which then were dried for 30 min at 60 °C and added to liquid scintillation medium. The radioactivity was measured by a Packard Tricarb Spectrometer. The experiments were done in quadruplicates and the SD were calculated.

Results. Previous experiments revealed that an incubation time of 60 min for 8-MOP is necessary to obtain maximum inhibitory rates of ^{3}H-TdR incorporation. Also, photoinactivation proceeded slowly during the hours following irradiation, so that after 24 h dose-effects could be measured (unpublished). Our results show a clear dose response curve



Semilog graph of ^{3}H-thymidine incorporation rates in 3T3 cells treated with various dosages of 8-MOP and UVA. Photoinactivation is complete at a phototoxic index (PTI), abscissa, of 6.

Effects of various concentrations of 8-MOP and different dosages of UVA light on ^{3}H-thymidine incorporation of 3T3 cells. Cells were irradiated 60 min after incubation of 8-MOP and the uptake of ^{3}H-thymidine was measured 24 h later. Data are given as mean (cpm of 4 separate cultures) ± SD

8-MOP (µg/ml)	J/cm^2 0	0.65	1.3	1.95	2.6
0	6276±859	6278±318	6100±1007	6558±304	6104±368
0.01	5597±756	6264±265	6612± 578	6620±553	6372±715
0.02	6120±348	5246±926	5317±1085	5936±863	5819±969
0.04	6528±298	5520±521	4798± 871	5688±614	5027±863
0.1	6404±804	4326±830	3982± 578	3422±497	3173±200
0.2	6206±490	3887±558	3916± 376	2953±509	2171±170
0.4	5658±146	3589±570	3590± 440	1919±407	1409± 88
1	5873±469	1981±363	1500± 335	480±219	336± 94
2	5900±190	1357±203	956± 182	185± 50	69± 25
4	6538±524	852±229	430± 159	30± 4	20± 2
10	6463±611	25± 5	17± 5	21± 3	18± 5

when the data are plotted individually for 8-MOP and UVA (table). In order to compare the effects of various 8-MOP concentrations with different exposure lengths of UVA, the individual data were plotted semilogarithmically as the product of psoralen concentration and UVA dose (figure). This product may be called the phototoxic index (PTI). As can be seen on the figure, the incorporation rates show a linear decrease with an increasing PTI. The minimal effective PTI value was approximately 0.01, whereas complete inhibition was found at a PTI of 10 (figure). The inhibitory range is therefore three orders of magnitude independent of the individual dose of light or psoralen used. The linear curve in the figure may be calculated by the following formula:

$$\text{cpm} = c \times \ln \frac{\text{J/cm}^2 \times \mu\text{g 8-MOP/ml}}{\text{PTI}_{max}}$$

Abbreviations:
c = constant relative to cell type under study
ln = natural logarithm
PTI_{max} = complete inhibition of ^{3}H-TdR incorporation

The results show that the inhibitory effect of psoralen and 8-MOP upon scheduled DNA synthesis in cultured cells is a product of the effect of psoralen and light. By this method, information on the photosensitizing effects of a given 8-MOP concentration and the applied UVA dosage can be obtained rapidly, e.g. the absolute sensitivity of these cells is obtained by determining the minimum PTI, whereas the relative sensitivity is shown by the slope of the curve.

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Hepatic UDP-glucuronyltransferase activity in acrylamide neuropathy[1]

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Summary. Acrylamide was found to be tissue selective in its toxicity. Doses which were clearly neurotoxic to rats were without effect on hepatic UDP-glucuronyltransferase, total hepatic protein or microsomal protein.

Endoplasmic reticulum (ER) in nerves becomes disordered in certain toxic neuropathies. Dorsal root ganglia and anterior horn cells exhibit freeing and dispersion of granular ER and polyribosomes in cats with acrylamide neuropathy[2]. Tubulovesicular profiles of ER become evident in the axoplasm of animals given vincristine[3] or bis-(N-oxopyridine-2-thionato) zinc II[4]. Amino acid incorporation into the nerves is depressed prior to pathological evidence of the neuropathy[5] and then becomes enhanced when the neuropathy is established[6].

Acrylamide is distributed in the total body water[7] and hence might be expected to exert a similar disruptive action on non-neural ER. Liver UDP-glucuronyltransferase (UDP-GT) from acrylamide intoxicated rats provides a readily accessible substrate upon which to assess this action since it is located in the ER, is affected by changes in its microenvironment[8-10] and is important in the metabolism of many xenobiotics.

Methods. Male Wistar rats (120–200 g) were given monomeric acrylamide dissolved in sterile saline (30 mg/kg s.c. daily for 14 days); control animals received an equivalent volume of saline. Animals were judged to be neuropathic when they exhibited splaying of the hindlimbs, motor paralysis and loss of body weight. On the 15th day, when neurotoxicity was evident, the rats were decapitated, their livers rapidly removed and chilled in ice-cold 1.15% KCl and microsomal fractions prepared as previously described[11].

UDP-GT activity was measured in microsomal fractions using either o-aminophenol (OAP) or p-nitrophenol (PNP) as substrates. Typical incubation mixtures contained in a final volume of 0.5 ml: 1-3 mg microsomal protein; 50 mM Tris-HCl buffer, pH 7.4; 5.0 mM $MgCl_2$; 0.5 mM of phenolic substrate and 5.0 mM UDP-glucuronic acid. Reactions were started by adding UDP-glucuronic acid and run for 15 min at 37 °C. OAP glucuronidation was estimated by the method of Dutton and Storey[12]. PNP glucuronidation was estimated by determining the disappearance of PNP as previously reported[11]. All determinations were run in duplicate including blanks, which contained all reactants

Table 1. Effect of chronic acrylamide treatment on hepatic UDP-GT activity. Rats were treated for 14 days with either acrylamide (30 mg/kg, s.c.) or an equivalent volume of saline vehicle. Hepatic microsomal fractions were prepared 24 h later and UDP-GT activity was determined

Treatment	UDP-GT activity* (nmoles/15 min/mg protein)	
	OAP	PNP
Control	4.0±0.39	26.2±4.9
Acrylamide	4.7±0.14	23.0±4.1

* The values are the mean ± SEM for 4 animals.

Table 2. Effect of chronic acrylamide treatment on liver weight and total and microsomal hepatic protein content. Rats were treated as described in table 1

Treatment	g liver/100 g b.wt*	Total protein (mg/g liver)*	Microsomal protein (mg/g liver)*
Control	4.57±0.07	231±3.6	11.4±0.5
Acrylamide	4.29±0.14	241±3.3	12.9±0.7

* The values are the mean ± SEM for 4 animals.