Inhibition of Nucleic Acids Synthesis in Ehrlich Ascite Tumor Cells by Irradiation in vitro in the Presence of Skin-Photosensitizing Furocoumarins

We have already reported that mouse Ehrlich ascite tumor cells lose their in vivo ability to induce tumors when irradiated at 365 nm (on its own inactive) in the presence of skin-photosensitizing furocoumarins (psoralens)[1]. These substances are so-called for their well-known capacity to produce cutaneous erythemas by action of long wavelength ultraviolet light[2].

It has been ascertained that they photoreact with nucleic acids, giving place to a C_4-cyclo-addition to the pyrimidine bases[3–5], behaving both as a monofunctional and bifunctional reagent[6].

Besides, Ehrlich ascite cells, irradiated at 365 nm in the presence of psoralen or 8-methylpsoralen, are able to induce protection in animals against graft of the tumor cells[7].

We have studied the consequences of the action of the skin-photosensitizing furocoumarins and therefore investigated the differences between treated and untreated cells.

We had previously demonstrated the formation of covalent linkages between psoralen-^{3}H and DNA by irradiation in vitro of Ehrlich ascite cells[8], and the behaviour of these as untreated tumor cells with regard to Wright's liquid, trypan blue and oxygen uptake[7].

In this paper we have studied nucleic acids synthesis and the furocoumarins psoralen, xanthotoxin (8-methoxy-psoralen), bergapten (5-methoxy-psoralen) and xanthotoxol (8-hydroxy-psoralen).

Cell suspensions ($2–3 \times 10^6$ cells/0.1 ml) in saline solution containing the furocoumarin were irradiated in Petri dishes with a Philips HPW 125 lamp (365 nm; irradiation intensity 1.07×10^{15} quanta/cm^2/sec, determined with a chemical actinometer[9]).

The cells, washed 3 times with ice-cold Krebs-Ringer phosphate buffer, pH 7.2, and suspended in the same buffer (4.5×10^6 cells/0.1 ml) were incubated for 30 min at 37 °C in the presence of the radioactive precursor (3.3 μCi/ml; thymidine-methyl-^{3}H, 2 Ci/mM; uridine-^{3}H, 6 Ci/mM; Radiochemical Centre, Amersham, England).

Inhibition of the nucleic acids synthesis in Ehrlich ascite cells after irradiation in vitro in the presence of skin-photosensitizing furocoumarins

Furocoumarin	μg/10^6 cells	Quanta $\times 10^{-18}$	Percent inhibition DNA	RNA
Psoralen	1	0	0	—
	1	2.93	70	—
	1	4.86	80	—
	1	9.73	93.5	—
	0.12	0	0	0
	0.12	1.46	20	5
	0.12	2.93	24	7
	0.12	4.86	35	10
	0.12	9.73	51	29
	0.12	19.4	68	71
Bergapten	0.12	0	0	0
	0.12	19.4	45	20
Xanthotoxin	0.12	0	0	0
	0.12	19.4	52	40
Xanthotoxol	0.12	0	0	0
	0.12	19.4	0	0

The cell suspensions, containing the furocoumarin, have been irradiated at 365 nm and thus incubated with thymidine-^{3}H or uridine-^{3}H; nucleic acids were extracted with NaCl 10% and the radioactivity determined.

After 3 washes with Krebs-Ringer phosphate buffer containing the unlabelled precursor ($5 \times 10^{-3} M$), the cells were treated with hot 80% aqueous ethanol for 5 min. Both nucleic acids were extracted with hot 10% sodium chloride solution, precipitated with ethanol and dissolved in 3 N ammonium hydroxide[10]. These solutions were used to determine the DNA content according to BURTON[11] and the radioactivity with a Beckman LS-150 liquid scintillation spectrometer using a modified Bray's solution[10,12] (efficiency 35–37%). The results were calculated on the basis of dpm/mg DNA. Specific activity of the controls was $13–16 \times 10^4$ dpm/mg DNA with thymidine-^{3}H and $22–29 \times 10^4$ dpm/mg DNA with uridine-^{3}H. The data are summarized in the Table, and are the average of various experiments.

A considerable inhibition of nucleic acids synthesis particularly with psoralen, which depresses the DNA synthesis even with small doses of UV-light, is noted. The RNA synthesis decreases only for relatively prolonged irradiation.

The long-wave UV-light by itself and treatment with furocoumarins in the dark have no effect. One must also note that xanthotoxol, free from skin-photosensitizing activity and not photoreacting with nucleic acids, is not able to inhibit incorporation of labelled precursors.

In conclusion, these data indicate that the observed inhibition of the nucleic acids synthesis in Ehrlich ascite cells is to be related to the capacity of the skin-photosensitizing furocoumarins to react by UV-irradiation with nucleic acids.

The results reported here are in agreement with those obtained by TROSKO and ISOUN[13], with 4.5′, 8-trimethylpsoralen and human amnion (AV_3) cells in culture.

Riassunto. La sintesi degli acidi nucleici nelle cellule del tumore ascitico di Ehrlich viene inibita dall'irradiazione a 365 nm in vitro in presenza di furocumarine fotosensibilizzatrici cutanee (psoralene, bergaptene e xantotossina).

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