Photoinactivation of Ehrlich Ascites Tumor Cells in vitro Obtained with Skin-Photosensitizing Furocoumarins

It is known that various biological effects may be exerted by the so-called skin-photosensitizing furocoumarins on different substrates upon irradiation at 3.655 Å[1-7]. By the term 'skin-photosensitizing' we wish to indicate the active furocoumarins, so distinguishing them from the inactive ones and from many other substances called 'photodynamic' which, upon irradiation, exert their biological effects with a mechanism completely different from that of furocoumarins; i.e. through a photooxydative process[8,9]. It is known, from research carried out in this Institute, that the photobiological effects of furocoumarins are oxygen-independent and are due to the formation, following irradiation, of a stable chemical linkage with DNA[10] by photocycloaddition to pyrimidine bases.

In the present paper we report on the inactivation of the Ehrlich ascites tumor cells (i.e. on the destruction of their tumor-producing capacity) obtained in vitro by irradiation at 3.655 Å in the presence of psoralen, xanthotoxin, or bergapten, 3 typical skin-photosensitizing furocoumarins.

Our experiments were carried out using mouse Ehrlich ascites tumor, cultured for many years in our laboratories by transplantation in albino Swiss mice. Concerning the characteristics of our tumor source, we can say that $2 \cdot 10^6$ cells, i.p. injected in a mouse, always produce ascites and death within 14 days. After 8 days the average quantity of ascites exudate is 5 ml, and it contains $8–20 \cdot 10^6$ cells/0.1 ml. Employing gradual dilutions, we have found that LD_{50} after 30 days corresponds to the injection of $2–5 \cdot 10^2$ cells/mouse.

The tumor cell suspensions were prepared by dilution of a pool of ascitic exudate with physiological saline, or with saline containing varying amounts of furocoumarin, up to a final concentration of $2 \cdot 10^7$ cells/ml. The final concentration of the furocoumarins was variable (see Table).

With the aim of irradiating the cells under sterile conditions and in thin layers, 3 ml of the prepared suspensions were pipetted into the horizontal arms of sterile L-shaped test tubes which were placed over crushed ice at a 15 cm distance from a Philips HPW 125 lamp and irradiated (3.655 Å) for 30 min (irradiation intensity: $4.2 \cdot 10^{15}$ hν/sec/cm²).

The tumor cells' viability was determined by i.p. injecting 0.25 ml of the suspensions, i.e. $5 \cdot 10^6$ cells, in 20 g Swiss mice. The injections were always made within an hour from time of collection of the exudate. During this time the tubes were constantly kept in contact with the crushed ice.

Control experiments, carried out under conditions identical with those reported in the Table, have indicated that keeping the cell suspensions in ice for an hour, as well as irradiating them (3.655 Å) for 30 min in the absence of furocoumarins and treating them with furocoumarins in the dark, does not modify the tumor-producing capacity of the cells. Also, irradiating in the presence of a skin-inactive furocoumarin (xanthotoxol, or 8-hydroxypsoralen) does not produce any inactivation of the tumor cells. Inactivation was obtained only by the contemporaneous action of both UV-radiation and the skin-active furocoumarins.

The results obtained are reported in the Table. They show that the i.p. injection of a suspension containing a number of tumor cells corresponding to 10^4 LD_{50} ($5 \cdot 10^6$) produces the death of all animals.

Death also occurred in several of the mice which were injected with cell suspensions irradiated in the presence of extremely small amounts of furocoumarins (Table); i.e. 0.0005 and 0.0025 μg of psoralen, 0.0025 and 0.005 μg of xanthotoxin and 0.0025, 0.005, 0.025 and 0.05 μg of

Influence of irradiation (3.655 Å) in the presence of furocoumarins on the tumor-producing capacity of Ehrlich ascites tumor cells

Furocoumarin concentration[a] μg/million cells	No. of treated mice[b]	Mortality[c] No.	%
Psoralen			
– (controls)	168	168	100
0.0005	8	6	75
0.0025	8	3	37
0.005	8	0	0
0.025	8	0	0
0.05	28	0	0
0.25	110	0	0
0.5	10	0	0
1	60	0	0
Xanthotoxin			
– (controls)	50	50	100
0.0025	8	8	100
0.005	8	6	75
0.025	8	0	0
0.05	8	0	0
0.15	24	0	0
0.25	8	0	0
Bergapten			
– (controls)	50	50	100
0.0025	8	8	100
0.005	8	8	100
0.025	8	1	12
0.05	20	7	35
0.15	24	0	0
0.20	8	0	0
0.25	10	0	0

[a] The irradiated suspensions contained $2 \cdot 10^7$ cells/ml. [b] The amount of tumor cells i.p. injected in a single mouse was $5 \cdot 10^6$ cells, equal to about $10^4 \cdot DL_{50}$. Such a dose produced the death of all control mice within 12 days. [c] Mice were observed at least for 60 days.

[1] L. Musajo and G. Rodighiero, Experientia *18*, 153 (1962).
[2] L. Musajo, G. Rodighiero and G. Caporale, Bull. Soc. Chim. biol. *36*, 1213 (1954).
[3] L. Musajo, Pure appl. Chem. *6*, 369 (1963).
[4] W. L. Fowlks, D. G. Griffith and E. L. Oginsky, Nature *181*, 571 (1958).
[5] M. M. Mathews, J. Bact. *85*, 322 (1963).
[6] E. L. Oginsky, G. S. Green, D. G. Griffith and W. L. Fowlks, J. Bact. *78*, 821 (1959).
[7] L. Musajo, G. Rodighiero, G. Colombo, V. Torlone and F. Dall'Acqua, Experientia *21*, 22 (1965).
[8] L. Musajo, G. Rodighiero and L. Santamaria, Atti Soc. ital. Patol. *5*, 1 (1957).
[9] L. Musajo and G. Rodighiero, Atti Accad. naz. Lincei Rc. *38*, 591 (1965).
[10] L. Musajo, G. Rodighiero and F. Dall'Acqua, Experientia *21*, 24 (1965).

bergapten always per 1 million cells. (After approximately 1 month a few mice died, having developed only solid tumors.)

On the contrary, mice treated with cell suspensions irradiated in the presence of slightly larger amounts of furocoumarins did not develop tumors and appeared to be in normal health.

We have ascertained that the cell suspensions contained the same number of cells prior to and following irradiation in the presence of furocoumarins. Irradiated cells, Wright stained and microscopically observed, had a morphological aspect identical to that of normal tumor cells.

In this in vitro anti-tumor effect, as well as in skin-photosensitization, psoralen is more active than xanthotoxin, while xanthotoxin is more active than bergapten.

We recall here that BELLIN, MOHOS and OSTER[11] obtained inactivation of a wide variety of tumor cells by irradiating them in the presence of several dyes used as sensitizers.

This photodynamic effect may be interpreted as caused by a photooxydative reaction. In our case, on the contrary, we know that oxygen is not involved, and we must attribute the disappearance of the tumor-producing capacity now observed to the same mechanism that we have suggested for the explanation of the other photosensitizing effects of furocoumarins[10]; that is, to the photochemical linkage of the furocoumarins to DNA[12].

Riassunto. Il liquido ascitico del tumore di Ehrlich, irradiato a 3655 Å in presenza di furocumarine «fotosensibilizzatrici cutanee» e cioè di psoralene o xantotoxina o bergaptene, perde la capacità di trasmettere il tumore nel topo.

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[11] J. S. BELLIN, S. C. MOHOS and G. OSTER, Cancer Res. *21*, 1365 (1961).

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Potentiation of Sympathetic Effects by a New Diphenylpropylamine Derivative[1]

The classical pharmacological tool for inducing supersensitivity in catecholamines is the administration of cocaine, which is a local anaesthetic and has marked central nervous system excitatory action, too. In this laboratory a new compound has recently been found in the course of a detailed research programme on diphenylalkyl derivatives which potentiates the effects of adrenalin and noradrenalin like cocaine but is devoid of other pharmacological actions of the latter. This compound is 1,1-*bis*-(4-amino-phenyl)-propyl-(3)-amine, to be referred to further as TK174.

TK174 increases the effects of exogenous catecholamines as well as that of the transmitter substance liberated by sympathetic nerve stimulation. The former was demonstrated in spinal cat and guinea-pig isolated vas deferens preparations. Pressor responses to i.v. administered catecholamines recorded by means of a mercury manometer on a smoked drum are tabulated in Table I. Some of the spinal cat preparations used (not included in Table I) had been subjected to bilateral adrenalectomy prior to blood pressure recordings. The enhancing influence of 2.5 mg/kg of TK174 on the pressor action of catecholamines was present, unaltered in these circumstances, too. Thus, the adrenal glands play no important role in this effect. The view of a peripheral action of TK174 is further confirmed by the results obtained on the isolated vas deferens, where TK174 potentiated the effect of noradrenalin (Table II) just as in vivo. Its activity is superior to that of cocaine.

[1] A detailed report will be published in the journal 'Drug Research' ('Arzneimittelforschung').

Table I. The influence of TK 174 on pressor responses to various drugs

Preparation used	Dose of TK174 mg/kg	Pressor substance	Dose/kg	No. of experiments	Pressor responses[a] recorded Before i.v. injection of TK174	After
Spinal cat	2.5	Adrenalin	0.25 µg	5	24.1 ± 4.5	56.8 ± 7.4
			0.5 µg	5	39.4 ± 7.0	86.4 ± 8.6
	2.5	Noradrenalin	0.25 µg	5	43.0 ± 7.4	85.2 ± 12.3
			0.5 µg	5	62.4 ± 11.5	105.8 ± 10.4
	2.5	Vasopressin	0.4 IU	8	46.1 ± 6.1	–
				9	–	56.8 ± .6.9
Narcotised rat	0.5	Tyramine	0.1 mg	5	23.2 ± 3.5	6.0 ± 1.7
			0.2 mg	6	31.3 ± 4.7	17.8 ± 4.0

[a] in mm Hg; mean values ± S.E.