Photosensitized Effects of Furocoumarins: the Possible Role of Singlet Oxygen

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The capacity of various furocoumarins to generate singlet oxygen in aqueous solution has been determined. The antiproliferative and the skin-photosensitizing activities of the same furocoumarins did not show any correlation with the capacity to generate the singlet oxygen, while these photobiological properties could be correlated with the capacity of furocoumarins to induce photolesions to the DNA.

Introduction

Furocoumarins are a group of naturally occurring or synthetic compounds which show interesting photobiological properties [1–3]. Some linear furocoumarins such as 8-methoxypsoralen (8-MOP), 5-methoxy psoralen (5-MOP) and 4,5′,8-trimethylpsoralen (TMP) are widely used for the photochemotherapy of skin diseases characterized by hyperproliferative conditions, such as psoriasis and mycosis fungoides [4–6].

The photobiological effects of furocoumarins have been generally attributed to their capacity to induce specific photolesions to the DNA molecule [1, 2, 7, 8]. More recent research, however, has clearly evidenced that furocoumarins are able to generate singlet oxygen by way of energy transfer from the excited state [9–12]. In this connection it has been suggested that some photobiological effects of furocoumarins such as skin photosensitization [13], the inactivation of lysozyme [9], of other enzymes [11, 14], and of *Escherichia coli* ribosomes [11] were mainly mediated by the singlet oxygen generated by the same furocoumarins.

To have an indication of the role of these two mechanisms in terms of biological consequences we have looked for a possible correlation between the capacity of a wide series of furocoumarins to generate singlet oxygen or to photobind to DNA *in vitro* and the capacity to provoke photobiological effects.

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Results

The evaluation of the capacity to generate singlet oxygen by various furocoumarins in aqueous solution has been carried out according to the method of Kraljic and Mohsni, *i.e.* using a system of imidazole plus *p*-nitrosodimethylaniline (RNO). The singlet oxygen formed reacts with imidazole forming a peroxide intermediate capable to induce the bleaching of RNO, which is evaluated spectrophotometrically [15]. The data obtained are reported in Fig. 1 in terms of percentage of RNO bleaching.

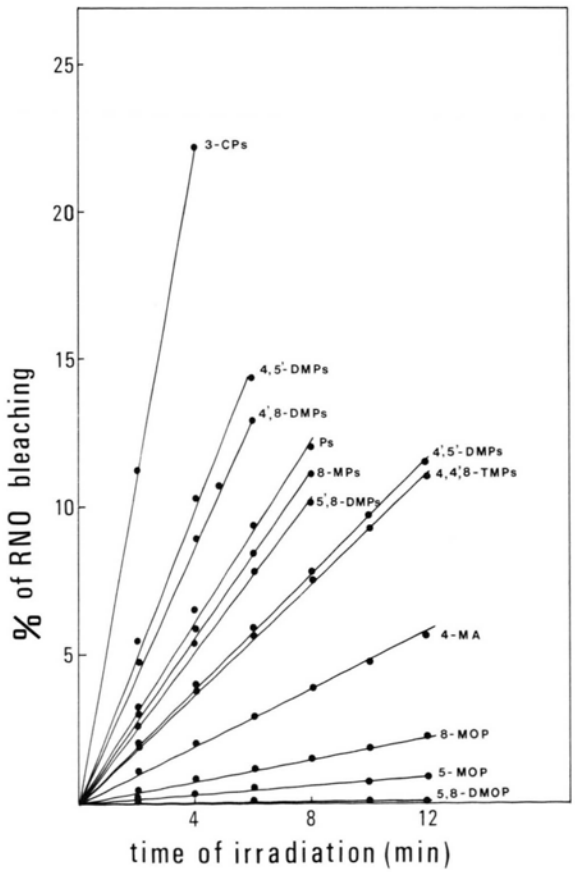

Fig. 1. Percent of RNO bleaching induced by the various furocoumarins as a function of time of irradiation; for abbreviations see Table.

Table I. Singlet oxygen generation capacity, antiproliferative and skin-photosensitizing activity shown by various furocoumarins.

Furocoumarin	Singlet oxygen generation		Antiproliferative activity[a]		Skin-photosensitizing activity (psoralen = 1)
	% of RNO bleaching after 4′ of irradiat.	Relative activity (psoralen = 1)	ID_{50}[b]	Relative activity (psoralen = 1)	
3-carbethoxy-psoralen (3-CPs)	22.2	3.41	247.00 [27]	0.004	inactive [16]
4,5′-dimethyl-psoralen (4,5′-DMPs)	10.3	1.58	1.59 [25]	6.16	2.7 [25]
4′,8-dimethyl-psoralen (4′,8-DMPs)	8.9	1.37	0.65 [19]	15.07	3.37 [18]
psoralen (Ps)	6.5	1.0	9.80 [19]	1.0	1.0 [18]
8-methylpsoralen (8-MPs)	5.85	0.90	1.04 [19]	9.42	5.4 [18]
5′,8-dimethylpsoralen (5′,8-DMPs)	5.4	0.83	1.82 [19]	5.38	3.37 [18]
4′,5′-dihydropsoralen (4′,5′-DMPs)	4.0	0.61	n. d.	–	inactive [18]
4′,4,8-trimethylpsoralen (4′,4,8-TMPs)	3.8	0.58	0.31 [19]	31.61	2.7 [18]
4-methylangelicin (4-MA)	2.0	0.31	24.00 [20]	0.4	inactive [20]
8-methoxypsoralen (8-MOP)	0.85	0.13	13.80 [27]	0.71	0.6 [18]
5-methoxypsoralen (5-MOP)	0.35	0.05	20.00 [19]	0.5	0.3 [18]
5,8-dimethoxypsoralen (5,8-DMOP)	practically inactive		n. d.	–	inactive [18]

[a] In terms of the extent of inhibition of the DNA synthesis in Ehrlich ascites tumor cells.
[b] ID_{50} = Irradiation dose necessary to provoke the 50% inhibition of the DNA synthesis in Ehrlich cells in the presence of the same amount of furocoumarin (4 μg/ml).
n. d. = not determined.

Similar results have also been obtained using a different method based on the DOPA decomposition caused by the singlet oxygen generated by the furocoumarins [13].

3-carbethoxypsoralen (3-CPs) lacking any skin phototoxicity [16] shows the highest production of singlet oxygen (see Table I, where in addition to the relative capacity to generate singlet oxygen by the furocoumarins their antiproliferative and skin photosensitizing activities are also reported).

Two other furocoumarins, unable to induce the skin erythema, *i.e.* 4′,5′-dihydropsoralen and 5,8-dimethoxypsoralen [17] show a quite different behaviour in terms of singlet oxygen generation.

Psoralen forms the singlet oxygen to almost the same extent as 8-methylpsoralen; this last furocoumarin, however, is much more active than psoralen in terms both of skin phototoxicity [18] and of antiproliferative activity [19]. 4-methylangelicin, lacking in skin phototoxicity and showing a relatively low antiproliferative activity [20] generates singlet oxygen to an extent higher than 8-MOP and 5-MOP, the most used drugs for photochemotherapy of hyperproliferative skin diseases and showing evident skin phototoxicity [17].

The data obtained indicate that no correlation between the capacity of various furocoumarins to generate singlet oxygen and the two photobiological activities taken into account can be observed.

If we consider, however, the capacity of furocoumarins to photobind to DNA we can observe that this property is well correlated with their antiproliferative activity on Ehrlich cells (see Fig. 2). In this Figure, in fact, the ID_{50} values (irra-

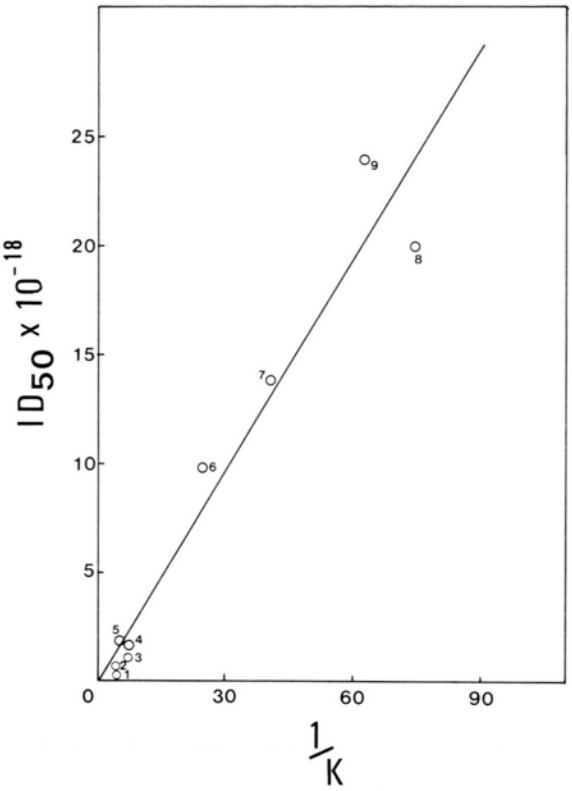

Fig. 2. Plot of ID_{50} (see text) against the reciprocal of the rate constant of the photoreactions (K) between various furocoumarins and the DNA. ID_{50} data from references [19, 20, 25, 27], rate constants from [20–22, 25]. The correlation is obtained by the least square method, according to the following equation: $ID_{50} = (-3 \pm 11)\ 10^{17} + (3.3 \pm 0.3)\ 10^{17}\ 1/K$; correlation coefficient $r = 0.971$. **1**: 4,4′,8-trimethylpsoralen; **2**: 4′,8-dimethylpsoralen; **3**: 8-8-methylpsoralen; **4**: 4,5′-dimethylpsoralen; **5**: 5′,8-dimethylpsoralen; **6**: psoralen; **7**: 8-methoxypsoralen (8-MOP); **8**: 5-methoxypsoralen (5-MOP); **9**: 4-methylangelicin.

diation dose which in the presence of the compound provokes the 50% inhibition of the DNA synthesis in Ehrlich ascites tumor cells [19], and which is inversely correlated with the antiproliferative activity) are plotted against the reciprocal of the rate constant values of the photoreactions of the various furocoumarins with DNA [20–22, 25]. This fact supports that this antiproliferative activity is mainly due to the photolesions induced in the DNA by the furocoumarins.

On the other hand a good correlation between the skin photosensitizing activity of various linear furocoumarins and their capacity to photoinduce interstrand cross-linkages in DNA has been reported previously [23].

These data seem to indicate that while the antiproliferative activity and the skin-phototoxicity of furocoumarins appear to be ascribed mainly to their capacity to photoinduce photolesions to the DNA, other photobiological activities such as the inactivation of enzymes or of ribosomes, are probably more connected with the formation of singlet oxygen.

Experimental

Furocoumarins

Psoralen and 5-methoxypsoralen (5-MOP) were extracted from fig-leaves and 8-methoxypsoralen (8-MOP) purchased from Chinoin. 8-methylpsoralen [24], 4′,8-dimethylpsoralen [24], 5′,8-dimethylpsoralen [24], 4,4′,8-trimethylpsoralen [24], 4′,5′-dihydropsoralen, 4,5′-dimethylpsoralen [25] and 4-methylangelicin [26] have been kindly given by Prof. G. Caporale, Dr. P. Rodighiero and Prof. A. Guiotto of our Institute. 3-carbethoxypsoralen (3 CPs) was kindly provided by Dr. E. Bisagni, Institute Curie, Orsay (France).

Singlet oxygen determination

The irradiations have been carried out by means of two HPW 125 Philips lamps, which emit over 90% at 365 nm [20].

According to Kraljic and Mohsni [15] all the solutions were prepared with double distilled water in phosphate buffer (pH 7.3 ± 0.1, 0.02 M) containing the furocoumarins (2.2×10^{-5} M), *p*-nitrosomethylaniline (RNO) (4×10^{-5} M) and imidazole (4×10^{-3} M). The solution in quartz cuvette, saturated by oxygen, was irradiated for increasing periods of time and the bleaching of the RNO was evaluated by the absorbance at 440 nm [15]. Control solutions containing furocoumarins in the presence of RNO or the imidazole in the presence of RNO, did not undergo modification in terms of absorbance of the RNO at 440 nm by irradiation at 365 nm.

Singlet oxygen was also determined according to de Mol and Beijersbergen van Henegouwen [13]: the aqueous solution in buffer (0.02 M phosphate buffer) containing the furocoumarin (2.2×10^{-5} M) and DOPA (1×10^{-5} M) saturated with oxygen, was irradiated for increasing periods of time and then the DOPA was evaluated fluorimetrically (λ_{ex} 284, λ_{em} 320) [13].

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