Skin Photosensitization and Cross-Linkings Formation in Native DNA by Furocoumarins

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Correlation between cross-linkages formation in native DNA and skin photosensitizing activity of furocoumarins.

Furocoumarins (psoralens) are a well known group of substances which show interesting photosensitizing properties on several biological systems [1–7]; the best known of these effects in sensitization of human and guinea-pig skin [2, 4, 8, 9], in the production of which the various furocoumarin derivatives show very different activities.

At present the photosensitizing effects of furocoumarins are attributed to their photobinding to DNA. These compounds photoreact with pyrimidine bases of native DNA forming both monofunctional photoadducts and bifunctional ones (cross-linkages).

At this point the question arises if monofunctional adducts and cross-linkings are or not equally effective in producing the skin-photosensitization. In this respect, the data obtained by Cole [10] studying the inactivation of *Escherichia coli* and bacteriophage λ by irradiation in the presence of psoralen stressed the greater importance of cross-linkages.

In past time, we have determined the initial rate constants of the total photobinding of several furocoumarin derivatives to DNA, without distinguishing the types of adducts formed [11]. Working with a small number of furocoumarins, we had found that the skin-photosensitizing potency of the various derivatives correlated well with their photobinding ability with DNA. Successively, however, by extending these studies to a larger group of furocoumarins, in particular to several methyl-derivatives of psoralen, this correlation was found to be no more present [12].

We have now determined for the photoreactions between several furocoumarins and native DNA the rate constant values of the cross-linkages formation. In particular we have irradiated at 365 nm 0.1% DNA aqueous solutions, containing 2 mM NaCl, in the presence of labelled furocoumarins for increasing periods of time, operating in the experimental conditions described elsewhere [11, 13].

The extent of cross-linkages formed after irradiation has been determined according to Lawley and Brookes [14] and the rate constant values have been calculated through a graphical method as previously described [11].

The results reported in Table I indicate that a good correlation between the rate constant values for cross-linkages formation by the examined furocoumarins and their skin-photosensitizing activities exists. This correlation suggests that the skin-photosensitization provoked by the various furocoumarins derives mainly from the amount of cross-linkages formed in native DNA. It appears therefore that bifunctional adducts, forming cross-linkings, are more effective than monofunctional one in provoking the skin-photosensitization.

Table I. Rate constants for cross-linkages formation in native DNA and relative skin-photosensitizing activity of furocoumarins.

Furocoumarins	Rate constants for cross-linkages formation [$\times 10^{-3}$ min^{-1}]	Relative cross-linkages formation rate (Psoralen = 100)	Relative skin-photosensitizing activity (Psoralen = 100)
Psoralen	3.79	100.00	100
8-methylpsoralen	11.77	310.55	540
5-methylpsoralen	8.53	225.06	450
5′,8-dimethylpsoralen	8.37	220.84	337
4,8-dimethylpsoralen	6.51	171.76	337
4,4′-dimethylpsoralen	2.92	77.04	60
4′,4,8-trimethylpsoralen	7.34	193.66	270
5′,4,8-trimethylpsoralen	6.92	182.58	270
3,4,8-trimethylpsoralen	2.96	78.1	54
Xanthotoxin	2.39	63.06	71
Bergapten	2.09	55.14	61

It is possible that this fact is due to a more easy recovery of the damage produced by the monofunctional adducts in comparison with that produced by bifunctional ones, as we have already observed in DNA of guinea-pig skin [15].

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