Psoralen photosensitization: damages to nucleic acid and membrane lipid components*

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For decades the therapeutic properties of psoralens were related to their photochemical reactions with DNA and RNA. Such approach, although fruitful in treatments, did not explain satisfactorily the way of healing of the multitude of diseases manifested through skin disorders.

The new research field presented in our review is directed to another target: the lipid components of the cell. The studies on the photobiology of phospholipids may help to elucidate the phototoxic and pigment inducing activity of psoralens.

Some plant extracts associated with exposure to sunlight have been used in popular medicine for as long as 5,000 years, for the relief of vitiligo, a skin disease characterized by lack of pigmentation. In the first decades of this century, the active components of these plants were identified as belonging to the class of furanocoumarins. Nowadays the naturally occurring psoralen is clinically used, as well as its 5- and 8-methoxy derivatives and the synthetic 4,5,8-trimethylpsoralen (cf. Scheme 1), although the mechanism by which they act in inducing pigmentation is still unclear.

In the 1960s, the search for this mechanism led Musajo, Rodighiero and their coworkers in Padova to discover that psoralens intercalate into the DNA helix and that, under UVA irradiation, they are able to photobind covalently the pyrimidine bases of DNA. This alkylation has a strong antiproliferative effect on cells, in that it causes inhibition of both DNA duplication and RNA/protein synthesis [1].

The discovery of this mechanism suggested to Parrish and other dermatologists at the Harvard Medical School in Boston the use of 8-MOP + UVA (320–400 nm), the so-called PUVA therapy, to treat diseases characterized by hyperproliferation of skin cells, in particular psoriasis [2].

The photoaddition of psoralens to DNA consists of a C(4)-cycloaddition reaction between either one of the two double bonds of the furanocoumarin or the 5,6 double bond of a pyrimidine base (mainly thymine) [3]. Thus, two different types of adducts can form: pyroneside and furan-side adducts (Scheme 2).

However, looking at the computer-simulated intercalation site, both double bonds of psoralen...
Psoralen

Scheme 1.

Psoralen are properly aligned with the double bonds of two thymine residues, if they are located on opposite strands of the macromolecule (Scheme 3). Inter-strand crosslinks may thus be formed, which is the most severe damage to DNA [4].

It has also been calculated that the axis of the helix is kinked by about 50 degrees at the crosslink site, thus impeding the action of enzymes [5].

Bifunctional lesions to DNA are not only thought to be more dangerous in terms of mutagenicity and risk of cancer induction [6], since the repair of crosslinks is generally more error-prone than that of monofunctional lesions. They are also often associated with strong phototoxicity: severe erythema and oedema appear after treatment with bifunctional psoralens. Thus, several attempts have been made to find pure monofunctional agents: on one hand, through chemical modification, either the pyrone or the furan ring have been made unreactive; on the other, angular furocoumarins were used (Scheme 4). In that case, the photoreactive double bonds of the drug were not properly aligned with those of thymines, and the probability of crosslink formation was low.

With a few exceptions, these monofunctional compounds were found not to be phototoxic and, in general, they were less mutagenic than psoralens, although they still retained their antiproliferative activity and, in some cases, good therapeutic effectiveness [7].

However, the photoinduced activity of furocoumarins is not limited to covalent addition. In fact, the drug molecule, once excited by UVA, can decay to its ground state, not only as a result of chemical reaction but also by transferring its energy to the surrounding molecules. Since the drug is mainly intercalated in the helix, the DNA bases and backbone are thus excited, just as it occurs when DNA is irradiated with shorter wavelength radiations, which are known to induce many deleterious effects on cell nuclei [8]. In this way, several kinds of lesions may be induced, such as thymine dimers [9], single- [10] and double-strand breaks [11], DNA-protein crosslinks [12], and so on. Moreover, energy transfer may occur from the

Scheme 2.
excited furocoumarin to molecular oxygen, yielding active forms of oxygen like singlet oxygen, hydroxyl radicals, superoxide anions, etc. [13]. These species have been shown to be dangerous to the DNA backbone and also to the bases which could have been oxidized [14].

These different chemical pathways, acting together, all contribute to the various photobiological effects of furocoumarins on nucleic acids.

However, as chemical and clinical research proceeded, it became evident that the target of psoralens is not only DNA. Proteins are also involved, although their role is still obscure [15], and cell membrane modification may account for the immunosuppressive effects observed during PUVA therapy [16]. Recent advances in the photopheresis [17] including irradiation of lymphocytes outside the patient’s body indicated that such mode of PUVA treatment may be a form of active immunotherapy. It was suggested that the reactions occurring in the cell surface DNA on lymphocytes [18] and in the phospholipids surrounding cell receptors [19] may be an important target of PUVA therapy.

The possible involvement of membrane lipids was investigated. When ethanol solution of furocoumarin was UVA-irradiated in the presence of an unsaturated fatty acid, a C_{4}\text{4}-cycloaddition took place between the 3,4 double bond of the former and one of the double bonds of the latter. The numerous adducts were isolated starting from various furocoumarins (psoralen, 8-MOP, 4,5,8-trimethylpsoralen, angelicin, 4,6,4'-trimethylangelicin, and some analogs: azapsoralens and heteropsoralens) and oleic (Scheme 5), linoleic, linolenic acids or their methyl esters [20–24].

One of the main problems encountered during these studies concerns the number of regio- and stereoisomers which can be formed; in fact, eight enantiomeric pairs of C_{4}\text{4}-cycloadducts are possible for each double bond [21]. Al-
though in the case of trimethylpsoralen photooxidized with oleic acid methyl ester only half of the isomers was shown to be found [23], several products with very subtle differences, namely in their chromatographic behaviour, still remain to be separated.

We were naturally interested in knowing whether these adducts play some biological role, and noted a certain structural similarity between them and 1,3-dioctanoylglycerol, (DAG, cf. Scheme 5), a synthetic diacylglycerol which was found to stimulate the activity of protein kinase C (PKC) [25], like physiological analogs derived from enzymatic cleavage of phospholipids in cell membranes.

We selected psoralen adducts with linoleic and linolenic acid and their methyl esters and tested them on human platelets: evident stimulation of PKC activity was found, measured in terms of phosphorylating activity [26].

Moreover, since DAG is able to induce skin pigmentation [27], we tested the ability of psoralen-fatty acid adducts to induce tyrosinase activity and melanin production by human melanocytes in culture [28]. Again, the adducts proved clearly effective, while the parent compounds — the fatty acid, the furcoumarin and its photolysis products — did not have any effect.

In vivo, unsaturated fatty acids are mostly esterified with glycerol giving rise to various classes of phospholipids. It is therefore probable that, if the photoaddition of psoralen to fatty acids occurs in cell membranes, it mainly involves these biomolecules. In this connection, we first studied egg yolk phosphatidylycholine (lecithin). However, the chromatographic separation of phospholipids is quite difficult, and it was even more difficult to discriminate between psoralen-lecithin-photoadducts and intact lecithins: indeed, the psoralen molecule is lipophilic and induces slight changes in the lipophilicity of lecithin. We were therefore obliged to follow a different strategy, detaching the fatty acid residue from the polar head and, since methyl esters have a better chromatographic behaviour than free acids, by submission of the photoacted lecithin to methanalysis instead of hydrolysis. The resulting solution was then compared by HPLC with ethanol solutions of psoralen irradiated in the presence of methyl esters of oleic, linoleic, linolenic and arachidonic acid: the correspondence of several chromatographic peaks suggested that C_{4}-cycloaddition had occurred in all the acid moieties [29]. Since the fatty acid content of the natural lecithin is very complex, some synthetic lecithins containing a single residue were studied, yielding clearer results. β-Linoleyl-γ-palmityl-α-phosphatidylycholine was also photoacted with psoralen, and the crude reaction mixture was analysed by mass spectrometry using the MALDI (Matrix Assisted Laser Desorption/Ionization) technique: besides the big peak of lecithin at m/z 758, the high-mass region of the spectrum revealed an evident signal at m/z 944, corresponding to the addition of one psoralen molecule (M_r 186) (cf. Scheme 6, unpublished results).

To approach the in vivo situation, the above procedures were successfully carried out in a more complex systems such as human lympho-
cytes: 8-methoxypsoralen and UVA light were applied to a lymphocyte culture; the extract of its lipid fraction was then methanolysed and analysed by reverse-phase HPLC. Due to the small amount of material available, UV detection was not useful, and tritiated 8-MOP was employed; radioactivity was found in the fractions belonging to the unreacted compound and in those of adducts with linoleic and linolenic acid methyl ester [30].

Epidermis of rats treated with 4,6,4'-trimethyl-langelicin plus UVA was examined in the same way, and extraction of lipids followed by methanolysis resulted in isolation of adducts with oleic and linoleic acids [31].

For decades, the photobiology of psoralens has been considered to be closely connected with their action on nucleic acids. Although this hypothesis did lead to successful therapeutic achievements, new research fields are now open and may better elucidate the role of other biomolecular targets, in particular the lipid components of the cell membranes, in the phototoxic and pigmentogenic activity of psoralens.

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REFERENCES


