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Photosensitization by drugs*

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Abstract: Certain drugs are known to elicit photosensitivity side effects. A satisfactory understanding of the involved mechanistic aspects is necessary to anticipate the photosensitizing potential. We have used tiaprofenic acid (TPA), a photosensitizing nonsteroidal antiinflammatory drug, to illustrate the methodology followed to address this problem. After studying the photophysical and photochemical properties of TPA, the attention has been directed towards the reactivity of its lowest lying π–π^* triplet with biomolecules. Photosensitized lipid peroxidation occurs by a mixed type I (radicals) and type II (singlet oxygen) mechanism. In the case of proteins, the photosensitized reactions include Tyr, Trp, and His photodegradation, protein–protein photocrosslinking and drug–protein photobinding. This involves direct quenching of the drug triplet by the amino acid residues (Tyr and Trp) or by oxygen, followed by singlet oxygen oxidation (His and Trp). With DNA, the studies have included comet assay, induction of single-strand breaks in supercoiled DNA, and reaction with 2'-deoxyguanosine and thymidine. Product studies, together with time-resolved measurements, have shown that the fastest reaction occurs with purine bases, by a mechanism involving both radical and singlet oxygen processes. The employed methodology can be of general use to investigate the mechanistic aspects of photosensitization by drugs.

PHOTOSENSITIVITY SIDE EFFECTS

The combined action of drugs and sunlight on patients can produce both desired and undesired effects [1]. Thus, PUVA-therapy (psoralenes plus UVA-radiation) has long been employed for the treatment of psoriasis, while porphyrins are currently being introduced for the photodynamic therapy (PDT) of cancer or other diseases. By contrast, there is also a significant number of reports indicating that a variety of drugs can elicit undesired side effects, such as phototoxicity, photoallergy, or photocarcinogenicity [2,3].

The photobiological risk associated with the use of drugs depends on environmental and individual factors (climate, height on the sea level, type of skin, etc.). On the other hand, the photosensitizing potential is enhanced in the case of topically administered drugs or when the field of application is dermatology or ophthalmology. Considering all these factors, in a number of cases it may be advisable to evaluate the photobiological risk of a new drug candidate before its introduction in the market [3].

The mechanistic approach to risk prediction

In order to anticipate the appearance of photosensitivity side effects, a mechanistic understanding of the involved phenomena is necessary. Absorption of sunlight by drugs leads to their excited states. These can proceed further to afford drug-derived reactive intermediates or, under aerobic conditions, reactive oxygen species. Any of the above short-lived chemical entities may be able to interact with biological

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substrates, ultimately producing photodamage. Thus, in a mechanistic approach the key questions are to determine which are the responsible light-absorbing chromophores, the intervening excited states, the reactive intermediates involved, the target biomolecules, and the reactions taking place [3].

A model group of drugs for photosensitization studies

A number of drugs are capable of inducing photosensitivity disorders either after topical or systemic administration [2,3]. In particular, the nonsteroidal antiinflammatory 2-arylpropionic acids deserve special attention because they induce such disorders more frequently than other types of drugs [4–6]. Tiaprofenic acid (TPA) is a member of this family, that has been found to be one of the most potent photosensitizers in a multicenter photopatch test trial [5,6]. Its structure is that of 2-(4-[2-benzoyl]thienyl)propionic acid. In the present article, TPA has been chosen as a model compound to show the type of studies that can be undertaken in order to assess the photosensitizing potential of a given drug and to illustrate the methodological aspects of the problem.

DRUG PHOTOPHYSICS AND PHOTOCHEMISTRY

The absorption spectrum of TPA in neutral aqueous medium exhibits two intense bands with maxima at 266 and 314 nm and a weak tail extending up to 380 nm. The two main bands are shifted to the blue with decreasing solvent polarity [7]. The fluorescence of TPA appears at 420 nm and is very weak; its lifetime is lower than 0.5 ns. The phosphorence emission presents a maximum at 520 nm and is also very weak at room temperature. On the basis of the above absorption/emission spectra, complemented with theoretical calculations, the first excited singlet state is n–π*, and its energy is 81 kcal mol^{-1}. By contrast, the lowest lying triplet state has a π–π* configuration, with an energy of 58 kcal mol^{-1}. The second excited triplet is of n–π* nature and lies 10 kcal mol^{-1} higher [7].

The photoreactivity of TPA is mediated by its lowest lying π–π* triplet state [7]. In aqueous medium, TPA is converted into decarboxytiaprofenic acid (DTPA), with a quantum yield of 0.25 at 25 °C [7,8]. The chromophore of TPA is maintained in the photoproduct; therefore, DTPA can mediate the same photosensitization processes elicited by the parent drug. Although DTPA is photostable in water, it undergoes photoreduction in hydrogen-donating solvents such as isopropanol. The resulting ketyl-type radicals lead to hydrodimers as final products. On the basis of temperature-dependence studies and theoretical calculations, it has been concluded that both photodecarboxylation and photoreduction proceed from the triplet state; they require an activation energy between 7 and 10 kcal mol^{-1}, which is essentially coincident with the energy difference between the two triplets. On the other hand, the lowest π–π* triplet states of TPA and DTPA are reactive towards phenols or indoles (used as models for the Tyr or Trp units of proteins). The process, which does not require thermal activation, occurs via electron transfer from the donor to the (D)TPA π–π* triplet, followed by proton transfer. The result is again formation of the same ketyl radicals generated after hydrogen abstraction [7].

The intermediates involved in the photochemistry of TPA and DTPA have been characterized by nanosecond laser flash photolysis [9]. Photoexcitation of TPA at 355 nm in aqueous medium leads to the lowest π–π* triplet with a very high efficiency (ca. 0.9). The triplet is detected by its transient absorption, with maxima at 380 and 590 nm. Its deactivation occurs in the microsecond timescale (lifetime 0.8 μs) and is dominated by a thermally activated spin-allowed process (activation energy barrier ca. 10 kcal mol^{-1}). At neutral pH, there is an adiabatic loss of carbon dioxide, leading to a triplet biradical anion. This intermediate undergoes intersystem crossing to give a long-lived decarboxylated carbanion, which is finally protonated. In the case of DTPA, a similar triplet is also detected upon laser flash photolysis [9]. This species is essentially unreactive in aqueous medium, and hence its lifetime (6 μs) is markedly higher than that of triplet TPA. However, hydrogen abstraction in isopropanol is demonstrated by the diminished triplet lifetime (3.2 μs) and detection of the ketyl radical, which absorbs at 350 and 390 nm and decays by a second-order kinetics due to dimerization.

The triplets of TPA and DTPA are quenched by oxygen at close to diffusion-controlled rate, suggesting formation of singlet oxygen. This has been confirmed by means of time-resolved near infrared emission at 1270 nm [10]. The Φ_Δ value found for TPA in acetonitrile is 0.62; this value decreases sharply upon addition of base, due to competition between singlet oxygen formation and photodecarboxylation from the carboxylate anion. Accordingly, the Φ_Δ of DTPA (0.57) does not exhibit such medium dependence.

PHOTOSENSITIZED LIPID PEROXIDATION

One example of the potentially damaging effects of photosensitizers to aerobic cells is lipid peroxidation [11]. Linoleic acid (LA) has been extensively used as a model probe molecule for the study of lipid peroxidation. Thus, it is known that type I (radical) peroxidation leads to four conjugated dienic hydroperoxides, namely 9-hydroperoxide-*trans*-10-*cis*-12-octadecadienoic acid (A), 9-hydroperoxide-*trans*-10-*trans*-12-octadecadienoic acid (B), 13-hydroperoxide-*cis*-9-*trans*-11-octadecadienoic acid (C) and 13-hydroperoxide-*trans*-9-*trans*-11-octadecadienoic acid (D). By contrast, type II peroxidation produces only two of them (A and B), along with significant amounts of two other nonconjugated isomers, namely 12-hydroperoxy-*cis*-9-*trans*-13-octadecadienoic acid and 10-hydroperoxy-*trans*-8-*cis*-12-octadecadienoic acid [11,12]. In this connection, the possible involvement of the two mechanisms in the LA peroxidation photosensitized by TPA and its major photoproduct, decarboxytiaprofenic acid (DTPA) has been investigated by a combination of product studies and time-resolved measurements [13–16].

Analysis (HPLC) of the photomixtures resulting from the (D)TPA-sensitized irradiation of aqueous LA reveals the formation of all four possible conjugated dienic hydroperoxides A-D. Only traces of the nonconjugated isomeric peroxides are detected [15,16]. According to laser flash photolysis experiments the rate constants for hydrogen abstraction from linoleic acid by the excited triplet states of TPA and DTPA are 2×10^5 M^{-1} s^{-1} and 3.2×10^5 M^{-1} s^{-1} respectively. These data, together with the known rate constants for oxygen quenching of the triplets and for the reaction of singlet oxygen with linoleic acid, lead to the conclusion that the ratio between hydrogen abstraction and singlet oxygenation for TPA and DTPA is 1.1 and 2.1, respectively. For comparison, the same ratio for benzophenone is higher than 10^3. Thus, it appears that in the case of (D)TPA the mechanism should be mixed type I/type II [15]. However, hydrogen abstraction is only the initiation step of a radical chain peroxidation, and hence each LA-derived radical may give rise to a high number of molecules of peroxidic product. As a consequence, type I mechanism is largely predominating [16]

PHOTOSENSITIZED REACTIONS OF PROTEINS

Drug-induced photoallergy is one of the adverse reactions developed as a consequence of the combined effect of drugs and sunlight [3]. From the mechanistic point of view, photoallergy involves covalent drug-protein photobinding (haptenization) leading to the formation of a photoantigen. The resulting photoantigen may trigger a hypersensitivity reaction of the immune system. Besides, the photosensitized modifications of proteins may also produce extensive structural changes associated with loss of biological function [17]. Hence, a precise knowledge of the involved active sites and reaction mechanisms is required to understand the phototosensitizing potential of a given drug. Unfortunately, the reactions of low-molecular-weight compounds (drugs) with large biomolecules (proteins) are very difficult to investigate. This is even further complicated by the low degrees of functionalization achieved in most cases.

In this context, the changes taking place upon irradiation of purified bovine serum albumin (BSA) in the presence of TPA have been thoroughly investigated as a model system for drug-photosensitized protein modifications [17]. Acid hydrolysis of the photolysates followed by amino acid analysis shows a dramatic decrease of His and Tyr with respect to nonirradiated controls. No effect is observed when BSA is irradiated in the absence of TPA. Under anaerobic conditions, the observed His and Tyr decrease

is much less pronounced. Although Trp does not survive acid hydrolysis, it can be directly analyzed before this treatment by monitoring the characteristic Trp fluorescence emission at 350 nm. Actually, a sharp decrease of this amino acid is also observed after irradiation in the presence of TPA, which is more pronounced under aerobic conditions. Similar results are obtained with the decarboxylated photoproduct DTPA.

In order to elucidate the mechanism of (D)TPA photosensitized degradation of amino acids, different phenols have been chosen as simple analogs of Tyr. Upon irradiation of the (D)TPA/phenol mixtures, the products formed are (D)TPA hydrodimers, (D)TPA/phenol cross-coupling products, and phenol dimers arising from oxidative coupling [17,18]. Thus, it appears that there is a formal hydrogen abstraction by the excited benzoylthiophene chromophore of (D)TPA from the phenolic hydroxy group, to give a radical pair. The observed products can be esily accounted for in terms of geminate radical coupling or escape followed by radical dimerization. Formal hydrogen abstraction is best explained as occurring from a hydrogen-bonded exciplex, via electron transfer followed by proton transfer. This is consistent with the π–π^* nature of the (D)TPA triplet state [19].

More quantitative information on the mechanism has been obtained from kinetic data. Flash photolysis of (D)TPA in methanol produces the excited triplets, whose reactivity can be investigated by means of systematic quenching experiments. Using the methyl esters of His, Tyr, and Trp as quenchers, it has been established that the most rapid reactions occur with Trp and Tyr (rate constants in the range of 10^9 M^{-1} s^{-1}). The analogous process with His is considerable slower (rate constant lower than 10^7 M^{-1} s^{-1}). Taking also into account the rate constants for triplet quenching by oxygen and for the reactivity of singlet oxygen with the different amino cids, it follows that Tyr is essentially a type I substrate, while His is oxidized by a type II mechanism. Both reaction pathways can operate in the case of Trp [17].

Formation of (D)TPA/phenol cross-coupling products and phenol dimers [18] suggests the possibility that, besides photodegradation of amino acid units, other chemical modifications of proteins such as drug/protein photobinding and protein photocrosslinking (through coupling ot two Tyr units) can occur. This has been confirmed by irradiation of BSA with radiolabeled (D)TPA. Subsequent polyacrylamide gel electrophoresis followed by protein staining and/or radioactivity scanning of the gel shows the formation of higher-molecular-weight protein aggregates, as well as incorporation of the radioactive drug to all the protein bands [17].

PHOTOSENSITIZED MODIFICATIONS OF NUCLEIC ACIDS

Exposure of living organisms to solar radiation may induce lethal mutagenic and carcinogenic effects associated with photodamage to DNA [20]. At wavelengths shorter that 290 nm, the absorbing species is DNA itself; however, in the UVA region (320–400 nm), most photobiological effects are mediated by photosensitizers. Hence, sunlight-absorbing drugs may play an important role in DNA photodamage [20,21].

In order to determine whether TPA is able to photosensitize cellular DNA damage, human fibroblasts have been irradiated with the drug and subsequently examined by means of the comet assay [22]. This has led to the observation that TPA actually photosensitizes DNA fragmentation inside cells. To assess the nature of the observed damage, plasmid supercoiled DNA has been UVA-irradiated in the presence of different concentrations of TPA. Above 25 μM, TPA is able to photosensitize single-strand breaks (SSB), confirming previous observations by Artuso *et al*. [23,24]. But more importantly, lower concentrations of TPA (0.5–2.5 μM), not able to produce direct SSB under the same experimental conditions, do photosensitize oxidative damage to DNA, as revealed by the combined use of excision-repair enzymes formamidopyrimidine glycosylase (FPG) or Endo III and gel electrophoresis. The fact that TPA-irradiated DNA is substrate of the two enzymes clearly indicates that both purine and pyrimidine bases are oxidized [22].

The excited triplet state of TPA is quenched by 2'-deoxyguanosine and less efficiently by thymidine (rate constants: 10^8 vs. 10^7 M^{-1} s^{-1}, respectively). As stated above, DTPA has the same active chromophore as TPA. This chromophore is able to photosensitize the oxidation of key nucleosides, giving a variety of photoproducts. In the case of 2'-deoxyguanosine, the product mixture is characteristic of mixed type I/type II mechanisms. Thymidine is less reactive under similar conditions, but it also decomposes to give a typical type I product pattern [22].

Such changes of nucleic acid bases might have, in principle, mutagenic potential. This issue has been investigated by means of repair-defficient strains of *Saccharomyces cerevisiae.* The results indicate that TPA, although it photosensitizes DNA oxidative damage causing genotoxicity, does not promote DNA recombination and thus does not induce adducts in the DNA of yeast cells. This, together with cell effectiveness in repairing oxidative damage, may explain the fact that no cell mutation/transformation is observed [22].

In summary, TPA photosensitizes DNA cleavage inside cells. This probably involves type I and/or type II oxidative damage of purine and, to a lesser extent, pyrimidine bases. Although this damage can result in genotoxicity, the risk of mutagenicity does not appear to be significant on the basis of *in vitro* data.

CONCLUSION

A detailed photophysical and photochemical study of the interaction between excited drugs and key biomolecules such as lipids, proteins, and nucleic acids (or their simple building blocks) is essential for the understanding and prediction of the photosensitization processes. In the case of the model compound tiaprofenic acid (TPA), the photobiological properties can be explained taking into account its benzophenone-like structure [25] with a lowest lying π–π^* triplet and a ca. 10 kcal mol^{-1} higher n–π^* triplet. Further efforts along this line are necessary in order to establish structure/activity relationships that may allow to predict the photosensitizing potential of new drug candidates before their introduction in the market.

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