Photosensitization by drugs: quinine as a photosensitizer

DOUGLAS E. MOORE, *Pharmacy Department, University of Sydney NSW 2006, Australia*

Recently it was reported (Moore 1977) that a number of drug molecules with near u.v. absorption are able to sensitize photooxidation in methanol solution. Some, e.g. phenothiazines and thiazides, are able to sensitize both Type I (free radical) and Type II (singlet oxygen) mechanisms. Others, e.g. tetracyclines, quinine, appear to act as precursors for singlet oxygen only. Such observations may have relevance to clinical photoallergic and photoxic reactions exhibited in the therapeutic use of these substances.

In extending the study to aqueous solutions, preliminary experiments indicate a much lower capability of many of these drugs to act as precursors for singlet oxygen (Moore & Tamat 1979). In aqueous solution the solubility of oxygen is about one-tenth the value in methanol; also the lifetime of singlet oxygen is reported to be significantly shorter in aqueous media (Merkel & Kearns 1972). As well as these factors, the nature and yield of the primary species formed on irradiation may differ from one solvent to another. For instance, chlorpromazine on irradiation in propan-2-ol forms the triplet state of the molecule in high yield, whereas in aqueous solution, the dominant primary products are the chlorpromazine cation radical and the hydrated electron (Davies et al 1976; Navaratnam et al 1978). Only the triplet state of the sensitizer molecule appears able to interact with dissolved molecular oxygen thus producing excited singlet oxygen followed by oxidation of suitable substrates (Spikes 1977a).

Of the drugs previously tested for photosensitizing ability in methanol (Moore 1977), quinine is the lone example which shows significant photosensitizing ability in aqueous solution. The polarographic oxygen electrode apparatus (Moore 1977) was used to measure the rates of oxygen depletion upon irradiation at 30 °C of buffered air-saturated solutions of quinine containing one of the following oxidizable substrates:

2,5-Dimethylfuran (Aldrich)—purified by twice distilling under vacuum; L-tryptophan (Sigma), uric acid, sodium xanthine (both from ICN Pharmaceuticals), theophylline, theobromine and caffeine (BDH) were all used as received. Control irradiations of solutions of these substrates in the absence of quinine recorded negligible oxygen uptake.

Buffers (0·05 M) were prepared in doubly-distilled water using analytical grade reagents as follows: pH 2·0–3·0, K_2SO_4–H_2SO_4; pH 3·5–5·5, sodium acetate-acetic acid; pH 6·0–8·0, sodium hydrogen phosphate; pH 8·5–9·5, tris(hydroxymethyl)aminomethane-HCl; pH 10–11, sodium bicarbonate-NaOH.

Both quinine hydrochloride (BDH) and quinine sulphate (Hopkin & Williams) were used yielding identical reaction rates per mole of drug moiety. In the procedure adopted, 1 ml of freshly prepared quinine solution in buffer was added to 50 ml of air-saturated buffer containing substrate. The mixture was quickly transferred to the reaction vessel and the oxygen uptake measured upon irradiation.

Dimethylfuran as substrate. Irradiation with near u.v. light of air-saturated aqueous solutions of 2,5-dimethylfuran containing quinine leads to a zero order uptake of oxygen, at a rate depending on the pH of the solution, as shown in Fig. 1. Over the range 0·25 to 2·5 × 10^{-5} M, the rate was linearly dependent on the quinine concentration, indicating the direct participation of quinine as a photosensitizer. In the absence of dimethylfuran, the oxygen consumption was very slight in comparison. In both cases the absorption spectrum of quinine in the reaction mixtures showed slight increases in absorbance following irradiation. Typically, at pH 6·0 the absorbance at 334 nm increased by about 5% while the smaller 280 nm peak increased by 10% when dimethylfuran was absent. In the same time period (10 min) when dimethylfuran was present, 80% of the oxygen in the solution was consumed and the absorbance at 280 nm increased four-fold, corresponding to the oxidation product of dimethylfuran. Guilbault (1973) has noted a decrease in fluorescence intensity of quinine solutions caused by the high intensity excitation lamps used in fluorimetry. Thus some small chemical changes in quinine are evident upon irradiation but these are minimal in relation to the photosensitizing capability.

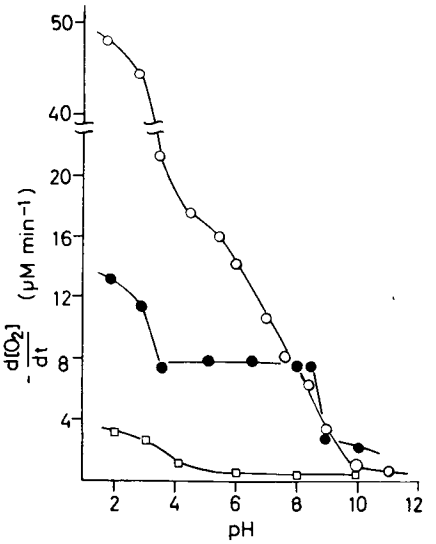

FIG. 1. Rate of oxygen uptake from air-saturated buffer solutions of varying pH at 30 °C, containing □ quinine 5·0 × 10^{-5} M; ○ quinine 5·0 × 10^{-5} M and 2,5-dimethylfuran 1·0 mM; ● quinine 5·0 × 10^{-5} M and L-tryptophan 1·0 mM.

The variation in photosensitizing capability with pH is clearly related to the state of ionization of the molecule. Quinine has pK_a values of about 4·2 and 8·3 (Perrin 1965) but the former is the more important, corresponding to protonation of the quinoline ring nitrogen. This protonation can be clearly seen from changes in the u.v. absorption spectrum. The λ_{max} changes from 334 to 348 nm from pH 6·0 to 2·0 and the absorption band becomes broader resulting in a more significant absorbance at 365 nm, where the medium pressure mercury lamp has maximum output. The fluorescence of quinine in aqueous solutions also depends on pH with maximum intensity in acidic solution. In fact, the change in intensity is such that quinine can be used as a fluorescent acid-base indicator for the pH range 4·0–5·0 (Guilbault 1973).

Of particular significance to biological systems is the observation that quinine fluorescence is strongly quenched by halide ions, and maximum fluorescence is measured in 0·1 M sulphuric acid solutions (Guilbault 1973). The photosensitizing capability is correspondingly quenched when chloride ion is added. Thus when a KCl-HCl mixture (0·05 M) was used for experiments at pH 2·0 and 3·0, the oxidation rate with dimethylfuran was approximately one-third the value obtained in K_2SO_4-H_2SO_4 mixtures of the same pH. The presence of 0·05 M KCl at pH 5·0 has a lesser effect, quenching fluorescence by about 15% with a similar decrease in the rate of the photosensitized reaction.

The addition of sodium azide (0·01 M) to the reaction mixture resulted in a complete quenching of the photosensitized reaction both at pH 3·0 and 5·0. This confirms the reaction as having a Type II (singlet oxygen) mechanism (Foote 1976).

Tryptophan as substrate. A detailed study of the photooxidation of various amino acids, including tryptophan, with methylene blue as sensitizer (Weil 1965) showed that the reaction occurred more strongly at a solution pH above the pK_a of the amino acid. In this region the neutral substrate, dimethylfuran, has indicated that quinine is less effective as photosensitizer. Nevertheless, photooxidation rates were recorded for tryptophan as shown in Fig. 1.

Chloride ion quenching was also observed for tryptophan reactions in a fashion similar to that described for dimethylfuran. Here the values are initial rates, since the oxygen uptake was not linear but diminished with time, as also observed by Weil (1965) with a manometric apparatus. The reaction was completely quenched by azide ion at pH 3·0 and 6·0 indicating the absence of a free radical (Type II) reaction. Straight & Spikes (1978) also studied the methylene blue photosensitized oxidation of tryptophan at pH 8·0 and found a similar curved rate of oxygen depletion, unaffected by the presence of radical scavengers.

The photooxidation of tryptophan is claimed to involve another excited oxygen species, superoxide ion (O_2^-), which leads to the formation of hydrogen peroxide (McCormick & Thomason 1978). Subsequent decomposition of this photoproduct to oxygen under the temperature and irradiation conditions employed here, may account for the fall in the rate of oxygen uptake from the initial rate values indicated in Fig. 1.

Table 1. Rates of oxygen uptake of purine derivatives (1 mM) upon irradiation in the presence of quinine (5×10^{-5} M) at 30 °C.

Substrate	Oxidation rate μM min^{-1} pH 7·5	pH 8·5	pH 9·5
None	0·84	0·58	0·22
Xanthine	3·5	2·4	0·81
Theophylline	3·2	2·3	0·82
Theobromine	0·86	—	—
Caffeine	0·88	—	—
Uric acid	4·1	2·8	1·03

Purine derivatives as substrates. A number of purine derivatives were tested as substrates as shown in Table 1. With these compounds the aqueous solubility was very low at pH <7; thus the reactions could be observed quantitatively only in systems where the photochemically less active form of quinine predominated. Nonetheless, zero order consumption of oxygen was observed, with inhibition by azide, for xanthine, theophylline (1,3-dimethylxanthine) and uric acid. The fact that theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine) were not oxidized indicates that the 7-position is the probable site of oxidation of xanthine.

The pH dependence of the quinine photosensitized reaction of xanthine, theophylline and uric acid above pH 7·5 showed the same general behaviour as observed with dimethylfuran and tryptophan.

Conclusion. Quinine is capable of acting as a generator of singlet molecular oxygen with an efficiency dependent on pH in the same way as its fluorescence intensity. The fact that it is able to act as a sensitizer for the photooxidation of the biological substrates tryptophan, xanthine and uric acid may have relevance to light induced adverse reactions to this drug. Although not specifically reported as giving rise to phototoxic reactions (Magnus 1976), there have been some cases of cutaneous flushing, skin rashes and visual impairment in hypersensitive individuals (Rollo 1975). Whether sunlight is involved in these reactions has not been made clear. However, quinine was reported as long ago as 1888 to be able to sensitize photodynamic damage in biological systems (Spikes 1977b).

Another relevant claim is that the photochemical decomposition of tryptophan is important with respect to ocular lens protein damage (Borkman et al 1977; Kurzel et al 1973). In this connection quinine acting as a photosensitizer could be exerting a detrimental effect.

September 21, 1979

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Inhibition of [^{3}H]diazepam binding by an endogenous fraction from rat brain synaptosomes

LEONARD G. DAVIS*, RONALD K. COHEN, *Missouri Institute of Psychiatry, University of Missouri-Columbia, School of Medicine, 5400 Arsenal Street, St. Louis, Missouri 63139, U.S.A.*

Benzodiazepine derivatives are among the most widely used sedative agents in the treatment of anxiety, tension, alcoholism, epilepsy, and insomnia. Recently, they have been shown to bind to specific receptors in the brain (Squires & Braestrup 1977; Möhler & Okada 1977a, b). The search for endogenous compounds that exist in the brain, and bind to these receptors, has met with some success. Two groups have independently reported that inosine and hypoxanthine can act as competitive ligands for the diazepam receptor (Skolnick et al 1978; Asano & Spector 1979). Both of these purines, however, have a low affinity for the receptor in that millimolar quantities are required for 50% inhibition of [^{3}H]diazepam binding. This concentration would be difficult to achieve under physiological conditions and, consequently, their role as endogenous ligands is unclear. Two additional compounds have been isolated from brain and reported to alter diazepam binding when tested in this radioreceptor assay. The first compound was shown to be a 15 000 amu (unified) protein that modulates the affinity of both benzodiazepines and GABA for their respective receptors (Guidotti et al 1978). The second compound, a protein of 30 000–70 000 amu, was isolated from porcine brain (Colello et al 1978). Because of its unprecedented size as a receptor ligand, this protein could be a proneurohormone. Conclusions reached from the above research reports have led us to further examine brain tissue for a compound(s) that can serve as the natural 'occupant' of the diazepam binding sites. Since diazepam receptors are found in the crude synaptosomal fraction (Mackerer et al 1978), and since brain membranes, when prepared for in vitro use, apparently maintain endogenous ligands bound to their specific receptors (Williamson et al 1978; Pert & Bowie 1979; Lippa et al 1978; Davis & Ehrlich 1979), we have utilized these biological enrichments of 'occupied' binding sites as the source of material in the search for endogenous diazepam ligands.

For the preparation of naturally occurring ligands, the cerebrums of decapitated rats ($n = 5$) were homogenized at 4 °C in 20 volumes of ice cold 0·32 M sucrose and centrifuged at 10 000 *g* min. The resulting supernatant was then centrifuged at 300 000 *g* min. This crude synaptosomal (P_2) fraction was then lysed by suspension in 5 volumes of 50 mM Tris-Cl buffer (pH 7·5), shaken for 30 min in the cold (4 °C), and frozen at −80 °C. After thawing, the suspension was again shaken in the cold for 30 min and then centrifuged at 10^6 *g* min. The supernatant obtained after these incubations and the freeze-thawing cycle was then lyophilized. The dried residue was dissolved in 0·01 M acetic acid and fractionated by gel filtration on a BioGel (Bio-Rad, Richmond, California) P-10 column. Evaluation of the chromatographic separation of the soluble components from the lysed synaptosomal fraction demonstrated that four major peaks could be distinguished by ultraviolet absorption. The four peak fractions were freeze dried. The fractionated material consisted of components in the molecular weight range of 1000 amu to those in excess of 20 000 amu.

Aliquots of the original material applied to the gel filtration column and each of the reconstituted fractions were tested for their inhibitory potential in the [^{3}H]-diazepam binding assay. The binding assay was performed with [^{3}H]diazepam (a generous gift from New

* Correspondence.