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Photochemical stability of biologically active compounds. III. Mefloquine as a photosensitizer

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Summary

The present investigation examined the photosensitizing potential of mefloquine in aqueous solution, including an evaluation of photooxidation properties, determination of reaction quantum yield for the decomposition of mefloquine, determination of fluorescence quantum yield and phosphorescence life time. Fluorescence and phosphorescence properties were evaluated for the degradation product formed initially in the photolysis of mefloquine.

Introduction

Drug photosensitivity refers to responses in tissues, mainly in the skin and eye, resulting from simultaneous exposure to certain chemicals and UV or visible radiation. A large number of drugs from different families of chemicals and different therapeutic classes have been implicated in photosensitivity reactions (Megaw and Drake, 1986). Compounds which have been developed for long-term medication and/or compounds which are administered in a high accumulative dosage are more likely to cause adverse photosensitivity responses in vivo. Changes in skin pigmentation and in the cornea and retina of the eye are side effects observed after long-term medication with antimalarials. These effects are possibly phototoxic reactions (Moore and Hemmens, 1982). To date, there are no reports indicating adverse photosensitivity reactions caused by mefloquine. This might, however, be ascribed to the lack of information on long-term treatment with mefloquine in humans. Mefloquine is still restricted in use for treatment of malaria, and should only be the drug of choice in areas with a high prevalence of multiple-drug resistance (Salako, 1985).

To some extent, the in vivo photosensitizing potential of a drug can be estimated from its in vitro photochemical behaviour (Oppenländer, 1988). The determination of the absorption spectrum of the drug will immediately establish

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whether it will absorb the optical radiation penetrating the viable layers of the skin (Oppenländer, 1988) or the various segments of the eye (Dayhaw-Barker and Barker, 1986). An apparent relation between the fluorescence quantum yield in vitro and photosensitizing ability in vivo has been observed for certain drugs (Moore and Hemmens, 1982). However, those compounds with long-lived triplet states are more likely to be photosensitizers, since most photochemical reactions originate from the triplet state (Roberts, 1988).

The reaction quantum yield of product formation or loss of starting material is important for predicting phototoxic reactions in vivo (Oppenländer, 1988).

The diversity in the structures of phototoxic compounds indicates that more than one photochemical mechanism is likely to be responsible for initiating phototoxic reactions in tissue. Because of the oxygen content of the blood, adverse reactions might be ascribed to photosensitized oxidation reactions. There are two mechanisms of photosensitized oxidation. The Type I mechanism is a free radical process and the Type II mechanism involves excited singlet molecular oxygen (oxygenation) (Spikes, 1977). For drugs that produce free radicals as well as singlet molecular oxygen, both mechanisms may be observed in the photooxidation.

The number of drugs found to be photolabile is increasing (Greenhill and McLelland, 1990). Because of the potential formation of toxic photoproducts from the drug itself, or by oxidation of biological molecules, characterization of the photochemical properties of drugs and drug formulations can no longer be ignored as a part of the preformulation. The present paper describes a study of the photophysical and photochemical properties of mefloquine, including fluorescence, phosphorescence and photooxidation. The primary photoproduct of mefloquine was also examined, and comparison was made with quinine, the structural analog with long-recognised antimalarial activity. The structures of these compounds are shown in Fig. 1.

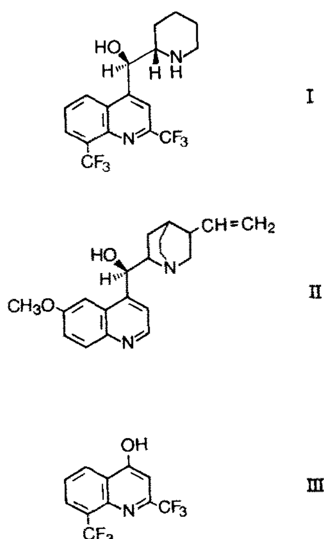

Fig. 1. Structural formulas of mefloquine (I), quinine (II) and the degradation product formed initially in the photolysis of mefloquine (compound III) (III).

Materials and Methods

Materials

The *erythro* racemate of mefloquine hydrochloride was provided by Hoffmann-La Roche, Basle. 2,5-Dimethylfuran and L-tryptophan were provided by Sigma and quinine sulphate was provided by Fluka.

Isolation of the initial photolysis product from mefloquine

50 mg mefloquine hydrochloride were dissolved in 500 ml water. The sample was exposed to light of wavelengths 280–350 and 400–510 nm, 120 W (Hereaus immersion lamp system, high-pressure mercury-halogen arc) for 2 h. The mixture was stirred during irradiation. After exposure to light, the sample was adjusted to pH 2 with 1 M HCl. The sample was extracted with 3 × 100 ml dichloromethane. The combined extracts were evaporated to dryness under vacuum. The residue was dissolved in 0.5 ml methanol. The degradation product was isolated by means of preparative TLC; stationary phase was silica gel (Merck), mobile phase was isopropanol/ammonium hydroxide (9 : 1). The sample was extracted from the silica gel with isopropanol. After centrifugation for 10

min at 2300 rpm, the sample was evaporated to dryness under vacuum and stored at $-20^\circ C$ pending further analysis. The molecular weight of the sample was determined by chemical ionization mass spectrometry, confirming the identity of the photoproduct (compound III) as 2,8-bis(trifluoromethyl)-4-hydroxyquinoline, as previously reported (Tønnesen and Grislingaas, 1990).

Quantitative TLC

The TLC system was as follows: stationary phase was silica gel 60 F_{254} (Merck), mobile phase was dichloromethane/methanol/glacial acetic acid (8 : 1 : 1).

For quantitation of mefloquine and degradation products a Shimadzu Dual-Wavelength Flying-Spot Scanner CS 9000 was used. Detection: (UV) 254, 283 nm; (fluorescence) excitation, 366 nm; emission, > 400 nm.

Reaction quantum yield determination

Actinometry was performed using the ferrioxalate chemical actinometer (Calvert and Pitts, 1966; Moore, 1987). Two cylindrical cells (4.5 ml) of spectroscopic grade silica were used. The reaction cells were immersed in a thermostat at $20 \pm 0.1^\circ C$.

The radiation source was a Photo-Irradiator consisting of a 900 W xenon arc lamp with an f3.4 monochromator (Applied Photophysics Ltd), operated at 320 nm with a bandwidth of 20 nm. Solutions of mefloquine in 0.05 M phosphate buffer, pH 7.4 were prepared. A concentration of 1×10^{-4} M mefloquine in buffer was used. Irradiation time was 1 min.

After irradiation, 3.0 ml of the sample were evaporated to dryness under vacuum. The residue was dissolved in 0.2 ml methanol and the sample was quantitated by means of TLC as described above.

Photooxidation measurements

The oxygen electrode was an Orion model 97-08-00. The cylindrical reaction vessel (37.5 ml) was constructed of spectroscopic grade glass (35% transmittance at 300 nm) with plane parallel faces 50 mm in diameter and 20 mm apart. The reaction vessel was immersed in a thermostat at $20.0 \pm 0.1^\circ C$. The reaction vessel was equipped with a magnetic stirrer. The Photo-Irradiator described above was operated at 320 nm with a bandwidth of 20 nm. The oxidisable substrates used were 2,5-dimethylfuran and L-tryptophan. Oxygen uptake rates were measured for mefloquine or quinine as photosensitizer over the pH range 3–10.

Stock solutions of mefloquine in methanol (2×10^{-3} M) and of quinine in 0.05 M H_2SO_4 (2×10^{-3} M) were prepared and further diluted to the appropriate concentrations in buffer. Buffers (0.05 M) were prepared in distilled water using analytical grade reagents as follows: pH 10, $NaHCO_3$-NaOH; pH 8–7, KH_2PO_4-NaOH; pH 5.5–4 sodium acetate-acetic acid; pH 3, Na_2HPO_4-H_2SO_4. The following conditions were studied for mefloquine and quinine: irradiation of drug alone (4×10^{-5} M in buffer); irradiation of drug plus 2,5-dimethylfuran (0.2 μl/ml). 2,5-Dimethylfuran was purified immediately before sample preparation by distillation. Irradiation of drug plus L-tryptophan (2×10^{-3} M); irradiation of L-tryptophan (2×10^{-3} M). Irradiation of plain buffer solution to correct for any possible background reactions.

Methanol was added to the quinine samples to equal the conditions of the mefloquine samples since the presence of organic solvents might influence the oxidation reactions.

Fluorescence quantum yields

Corrected fluorescence excitation and emission spectra of mefloquine, quinine and the isolated photodecomposition product from mefloquine (compound III, Fig. 1) were recorded from a Perkin-Elmer LS 50 luminescence spectrometer. Fluorescence quantum yields were determined as a function of pH for solutions of mefloquine, quinine and compound III by comparison with quinine dissolved in 0.05 M H_2SO_4 ($\phi_{fl} = 0.55$) as described by Calvert and Pitts (1966).

Stock solutions of mefloquine (2×10^{-5} M) and quinine (2×10^{-5} M) were prepared in methanol and diluted in buffer to concentrations 3×10^{-7} and 2×10^{-7} M for mefloquine and quinine, respectively. This is equivalent to an absorbance in the range 0.0008–0.001 at the excitation wavelengths used.

The isolated degradation product from mefloquine was dissolved in methanol and further diluted in buffer to a calculated absorbance of 0.001 at the excitation wavelength.

Excitation wavelength for mefloquine was 316 nm over the entire pH range. The emission spectra were recorded from 330 to 580 nm. Excitation wavelength for quinine was 330 nm at pH 10–4. Emission spectra were recorded from 340 to 580 nm. When quinine was dissolved in sulphuric acid and at pH 3, an excitation wavelength of 344 nm was used. The emission spectra were recorded from 360 to 580 nm.

Excitation wavelength used for compound III was 351 nm. The emission spectra were recorded from 370 to 600 nm. The buffer solutions (pH 3–10) were prepared as described previously.

UV-visible absorption measurements were made using a Shimadzu UV-260 UV-Vis recording spectrophotometer.

Phosphorescence lifetimes

Phosphorescence excitation and emission spectra of mefloquine, quinine and compound III were recorded in corrected mode from a Perkin-Elmer LS 50 luminescence spectrometer at 77 K. Solutions of mefloquine (1×10^{-5} M) and quinine (2×10^{-5} M) in ethanol were prepared. A solution of the isolated photolysis product was prepared to give an absorbance of 0.1 at the excitation wavelength. The wavelengths used for the calculation of phosphorescence lifetimes were as follows: (mefloquine) excitation, 285 nm, emission, 503 nm; (quinine) excitation, 344 nm; emission, 503 nm; (compound III) excitation, 285 nm; emission, 501 nm.

Results and Discussion

Reaction quantum yield

Mefloquine is photolabile at wavelengths relevant to biological conditions of the skin and the eye (Tønnesen and Grislingaas, 1990). The reaction quantum yield of the photodecomposition of mefloquine in buffer pH 7.4 irradiated at 320 nm was found to be 0.17 ± 0.01. In quantum yield determinations, the change in drug concentration should not exceed 15% during the irradiation in order that the photodecomposition remains linear with the irradiation time (Moore, 1987). For mefloquine under the conditions applying here, this meant using an irradiation time of only 1 min.

Fluorescence quantum yields

The fluorescence quantum yields for mefloquine, quinine and compound III as a function of pH, and the rate of oxygen uptake under various conditions are given in Table 1. The quantum yields are calculated relative to quinine in 0.05 M H_2SO_4, taken as 0.55. The fluorescence quantum yields for mefloquine in aqueous solutions are low compared to quinine. Various pK_a values for quinine are reported in the literature. The pK_a value for the quinoline N is found to be in the range pH 4–5 and the pK_a value for the quinuclidine N is found to be approx. 9 (Roth et al., 1985; Merck Index, 1989). The value of 8.6 given for mefloquine (Florey, 1985) relates to the piperidine nitrogen. It would be expected that the pK_a value of the quinoline nitrogen in mefloquine is about the same as for quinine.

The fluorescence of quinine in buffer is dependent upon the pH of the solutions, showing a maximum intensity at low pH. The dip observed at pH 4 might be an aberration due to the ionization process.

The fluorescence of mefloquine shows nearly the same pH dependence as observed for quinine, with a dip at pH 5.5. At pH 10, the fluorescence of mefloquine is increasing. The fluorescence quantum yields for compound III are significantly higher than for the parent compound mefloquine. The quantum yield shows little difference when calculated at pH 4 or pH 7. A lower value for the quantum yield is obtained at pH 10, corresponding to the deprotonation of the hydroxyl group.

Photosensitized oxidation

In order to compare the rates of photooxidation sensitized by mefloquine and quinine, correction has to be made for the differences in absorption spectra between the two compounds, and between different ionization states of the same compound. Thus, the rates were normalised to a constant amount of light absorbed, calculated from

TABLE 1

Spectral characteristics and photooxidation rates of mefloquine, quinine and compound III

Each result is the mean of 5 determinations.

Drug	Medium	λ_{abs} (nm)	ϵ (l mol^{-1} cm^{-1}) [a]	λ_{em} [b] (nm)	ϕ_{fl}	Oxygen uptake (μmol min^{-1} l^{-1}) [c]		
						Drug alone	Drug + DMF	Drug + L-tryptophan
Mefloquine	pH 10	317, 286	2900	375	0.018	0.75	2.90	3.14
	pH 8	316, 282	3240	377	0.015	0.73	3.34	1.79
	pH 7	316, 302, 282	3340	377	0.017	0.32	4.13	2.02
	pH 5.5	316, 302, 285	3250	375	0.012	0.09	4.51	1.49
	pH 4	316, 303, 283	3100	373	0.024	0.26	2.11	0.61
	pH 3	316, 303, 284	3080	373	0.022	0	9.95	2.47
Quinine	pH 10	326, 279	4100	368	0.040	0.07	0.43	0.53
	pH 8	330, 278	4550	370	0.386	0.16	3.97	1.45
	pH 7	331, 278	4300	367	0.463	0.23	5.85	2.16
	pH 5.5	331, 279	4300	370	0.482	0.23	6.75	2.06
	pH 4	334, 242	4300	368, 436	0.375	0.09	6.07	1.35
	pH 3	344, 317, 250	4800	433	0.547			
	0.5 M H_2SO_4	347, 317, 250	5100	440	0.55			
Compound III	pH 10			460	0.060			
	pH 7			467	0.150			
	pH 4			467	0.156			
	methanol	350, 335, 242						

[a] Calculated at the excitation wavelengths used in the determination of fluorescence quantum yields: mefloquine, 316 nm; quinine, 330 nm, pH 10–pH 4; 344 nm, pH $\leqslant$ 3; compound III, 351 nm.

[b] Values obtained from corrected fluorescence emission spectra, referred to quinine in 0.05 M H_2SO_4.

[c] Oxygen uptake rates were measured for irradiation at 320 nm with 20 nm bandwidth and normalised to constant light absorption by the sensitizers.

the area under the absorption curve determined across the bandwidth (20 nm) of the irradiation used. The oxygen consumption of solutions of the pure drugs is low in the absence of substrates, indicating that mefloquine and quinine do not sensitize their own oxidation to a significant extent. In alkaline solutions of pure mefloquine, however, the oxygen consumption is increasing compared to quinine. 2,5-Dimethylfuran (DF) and L-tryptophan (Trp) were used as substrates for photooxidation. DF is a strong indicator of the participation of singlet molecular oxygen with the advantages of adequate aqueous solubility, no spectral overlap with the irradiation range and pH independent reactivity (Moore and Hemmens, 1982). Trp is representative of amino acids which react with singlet oxygen (Matheson and Lee, 1979) although the photooxidation involves the production of superoxide anion and the reaction occurs more readily at alkaline pH (McCormick and Thomason, 1978). In the neutral form which predominates at pH 10, mefloquine is clearly a more efficient sensitizer than quinine for the photooxidation of both 2,5-dimethylfuran and L-tryptophan. In the singly protonated form the two compounds have about the same sensitizing potential. It would appear that the capacity to act as a generator of reactive oxygen species is dependent on pH in the same way as the fluorescence intensity, showing a dip at pH 4–5.5 corresponding to the dissociation of the quinoline N.

Phosphorescence

Compounds with long-lived triplet states are likely to have good photosensitizing abilities in vivo (Roberts, 1988). The lifetime of a molecule in

the excited triplet state is related directly to the phosphorescence lifetime. Quinine has a very weak phosphorescence, and the lifetime was found to be about 0.4 ms (deaerated glassy ethanol solution at 77 K). Compared to quinine under the same conditions, mefloquine has a 4 times stronger phosphorescence with a lifetime of 3.5 ms (deaerated glassy ethanol solution at 77 K). Since oxygen is an efficient quencher of triplet states, the phosphorescence of mefloquine is dependent upon the oxygen content of the solvent. When the sample of mefloquine in ethanol was deaerated under vacuum, a 5–10 fold increase in phosphorescence intensity was observed, compared to an air-saturated sample.

Because the compounds as salts were dissolved in ethanol, it can be assumed that it is the monoprotonated species being studied. The emission wavelength of approx. 500 nm corresponds to 237 kJ mol^{-1} as the energy level of the triplet state. Compound III is strongly phosphorescent. The phosphorescence lifetime was determined to be 10.9 ms (deaerated glassy ethanol solution at 77 K). A 10–20 fold decrease in phosphorescence intensity is observed in an air-saturated sample.

Degradation product fluorescence

As described previously, some of the photodecomposition products formed from mefloquine are strongly fluorescent compared to the parent compound, leading to an increase in fluorescence intensity of an aqueous solution of mefloquine as a function of irradiation time. An increase in fluorescence intensity of about 10% is observed after a sample of mefloquine is irradiated for only 15 s in direct sunlight. This seems to be due to the formation of the fluorescent degradation product of mefloquine, identified as 2,8-bis (trifluoromethyl)-4-hydroxyquinoline (Fig. 1, compound III). The identity of this compound has been determined previously (Tønnesen and Grislingaas, 1990), and was reconfirmed in this work by mass spectrometry.

The initial decomposition product (compound III) can easily be detected by TLC (fluorescence detection) in a sample of mefloquine after irradiation only for 5 min (Hereaus Immersion Lamp System). A scan of a thin-layer chromatogram of an aqueous sample of mefloquine irradiated for 4 h is shown in Fig. 2. Under the given experimental conditions, mefloquine is non-fluorescent. The area of the peak corresponding to compound III is calculated to be less than 1000 when detected at 254 nm. By assuming that the absorbances for mefloquine and compound III are equal at 254 nm, a peak area of 1000 corresponds to a concentration of compound III of approx. 10^{-7} M. When fluorescence detection is used, the same sample gives a peak area of 38 200. This clearly demonstrates the strong fluorescence of compound III in this TLC system, and the utility of the method for the early detection of photodegradation of mefloquine.

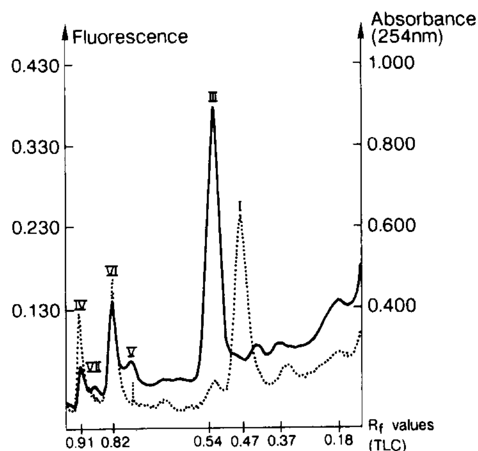

Fig. 2. Scan of a thin-layer chromatogram of an aqueous sample of mefloquine (pH 7.4) irradiated for 4 h. Chromatographic conditions are given in the text. (———) Fluorescence detection (Ex., 366 nm; Em., > 400 nm). (·······) UV absorption detection (254 nm) Peak I, mefloquine; peak III, compound III; peaks IV–VII were identified previously (Tønnesen and Grislingaas, 1990).

Free radical activity

The ability of mefloquine to generate free radicals on irradiation was tested by photopolymerisation experiments. Mefloquine was irradiated with acrylamide in deaerated aqueous solution at pH 7.0 in a dilatometric apparatus (Moore and Hemmens, 1982). No detectable reaction was observed for mefloquine in contrast to a small but measurable rate of polymerization initiated by quinine, and a significant rate for chloroquine due to ho-

molysis of the C-Cl bond (Moore and Hemmens, 1982). The C-F bonds in mefloquine are much more stable, and the energy of the triplet state insufficient to generate homolysis, in contrast to many chlorine-containing drugs, such as diclofenac (Moore et al., 1990).

Conclusion

The photosensitizing properties of mefloquine in aqueous solutions are exhibited principally through triplet state energy transfer to form singlet oxygen. These properties are observed also in a pH range corresponding to physiological conditions. The ability of mefloquine to act as a sensitizer for the photooxidation of L-tryptophan is important with respect to possible photodamage to the eye caused by this drug (Andley, 1987).

The photosensitizing potential of mefloquine and its primary photodegradation product (compound III) are further emphasized by the results obtained from phosphorescence measurements. Compared to quinine these compounds have long-lived triplet states. The phosphorescence is easily quenched by oxygen. In living tissues there is sufficient oxygen for photooxidation to occur. The relatively long-lived triplet states of mefloquine and compound III are therefore likely to cause the formation of excited oxygen species or other free radicals in biological systems as well as in aqueous solutions. In view of their ability to sensitize photooxidation and their fluorescent/phosphorescent properties, both mefloquine and compound III are good candidates for potential photosensitizers in vivo. Trace amounts of compound III might easily be formed in vitro due to various storage conditions of mefloquine tablets, or in vivo. The photochemical behaviour of compound III should therefore not be neglected.

Interaction of oxidation-sensitive amino acids or structural proteins with the excited states of mefloquine and compound III, are aspects worthy of further investigation. Binding to tissues is an important parameter to determine, since binding would increase in vivo retention time and potentially modify the photophysical and photochemical properties of these compounds.

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