Photoreactivity of biologically active compounds. XIX: Formation and reactivity of excited states and free radicals from primaquine

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Abstract

The formation and reactivity of excited states and free radicals from primaquine was studied in order to evaluate the primary photochemical reaction mechanisms. The excited primaquine triplet was not detected, but is likely to be formed with a short lifetime (< 50 ns) and with a triplet energy < 250 kJ/mol as the drug is an efficient quencher of the fenbufen triplet and the biphenyl triplet, and forms $^1O_2$ by laser flash photolysis ($^{PQ}\Phi_\Delta = 0.025$). Primaquine photoionises by a biphotonic process and also forms the monoprotonated cation radical ($PQH^{2+}\bullet$) by one electron oxidation by OH• ($k_q = 6.6\bullet10^9\ M^{-1}s^{-1}$) and $Br_2\bullet^-$ ($k_q = 4.7\bullet10^9\ M^{-1}s^{-1}$) at physiological pH, detected as a long-lived transient decaying essentially by a second order process ($k_2 = 7.4\bullet10^8\ M^{-1}s^{-1}$). $PQH^{2+}\bullet$ is scavenged by O_2, although at a limited rate ($k_q = 1.0\bullet10^6\ M^{-1}s^{-1}$). The reduction potential (E°) of $PQH^{2+}\bullet$ / PQH^+ is < +1015 mV. Primaquine also forms $PQH^{2+}\bullet$ at pH 2.4, by one electron oxidation by $Br_2\bullet^-$ and proton loss ($k_q = 2.7\bullet10^9\ M^{-1}s^{-1}$). The non-protonated cation radical ($PQ^+\bullet$) is formed during one electron oxidation with $Br_2\bullet^-$ at alkaline conditions ($k_q = 4.2\bullet10^9\ M^{-1}s^{-1}$ at pH 10.8). The estimated pK_a-value of $PQH^{2+}\bullet$/ $PQ^+\bullet$ is $pK_a \sim 7$-8. Primaquine is not a scavenger of $O_2\bullet^-$ at physiological pH. Thus self-sensitization by $O_2\bullet^-$ is eliminated as a degradation pathway in the photochemical reactions. Impurities in the raw material and photochemical degradation products initiate photosensitized degradation of primaquine in deuterium oxide, prevented by addition of the $^1O_2$ quencher sodium azide. Photosensitized degradation by formation of $^1O_2$ is thus important for the initial photochemical decomposition of primaquine, which also proceeds by free radical reactions. Formation of $PQH^{2+}\bullet$ is expected to play an essential part in the photochemical degradation process in a neutral, aqueous medium.

Keywords

Primaquine, photoreactivity, free radicals, excited states, pulse radiolysis, laser flash photolysis

Abbreviations

Abs	Absorption at given wavelength
BP	Biphenyl
Em.	Emission wavelength
Ex.	Excitation wavelength
E°	Reducion potential
FEN	Fenbufen
k_1	First order rate constant
k_2	Second order rate constant
k_{obs}	Observed rate constant
k_q	Bimolecular quenching rate constant
k_{ref}	Rate constant of reference compound
M	Molar per litre
PQ	Primaquine
PQox	Oxidized degradation products of primaquine
$^{PQ}\Phi_{\Delta}$	Quantum yield of $^1O_2$-formation sensitized by primaquine
$^{PN}\Phi_{\Delta}$	Quantum yield of $^1O_2$-formation sensitized by perinaphtenone
Sens	Photosensitizing impurities
TRPH	Tryptophan
TYR	Tyrosine
ϵ	Molar absorption coefficient
λ	Wavelength
λ_{max}	Absorption maximum
τ	Lifetime of transient
Φ_F	Fluorescence quantum yield
τ_F	Fluorescence lifetime

1. Introduction

The 8-aminoquinoline primaquine is an antimalarial compound that acts as a tissue schizontocide. The drug is mainly used curatively to eliminate latent liver forms of *Plasomdium ovale* and *P. vivax*, since severe haematological adverse effects (methaemoglobinaemia and haemolysis) prohibit use for general prophylaxis [1]. Primaquine is a photolabile compound, with a ground state absorption maximum at 350 nm and extension of the absorption spectrum towards 430 nm under physiological conditions. The drug decomposes photochemically into several degradation products at physiological pH, by oxygen dependent reaction mechanisms [2-4]. Primaquine forms an intramolecular hydrogen bond, which seems to be essential for chemical and biological properties, including photochemical stability and fluorescence. Photostability in protic media is well correlated with fluorescence properties, and quenching by breaking of the intramolecular bond and protonation of the quinoline nitrogen in the excited singlet state appears to be an important factor influencing photochemical stability of primaquine in pure, protic media [5].

Primaquine is an *in vitro* photosensitizer, inducing photohaemolysis, photopolymerization of lens proteins and photoreduction of cytochrome C [3, 6, 7]. Free radicals (OH• and O_2•-) that are formed in the photochemical reactions are important mediators also in the *in vitro* oxidation of haemoglobin induced by primaquine [8, 9]. The haematological adverse effects observed after medication with primaquine can (partly) be ascribed to photochemical reactions of the drug *in vivo*, taking into account that Caucasian skin transmits optical radiation above 320 nm [10].

The present work was undertaken to identify and quantitatively characterize the primary transient excited states and free radicals from primaquine, with the aim to obtain further knowledge on the kinetics and mechanisms of primaquine photodecomposition.

2. Materials and methods

Primaquine diphosphate (98-99 % pure) was purchased from Sigma-Aldrich. Primaquine-base was produced by extraction with $CHCl_3$ from an alkaline aqueous solution of the phosphate salt. The water used was deionised and purified. D_2O (99.9 % atom D) was purchased from Sigma-Aldrich. All other chemicals and organic solvents were of p.a. grade.

2.1. Preparation of samples for laser flash photolysis and pulse radiolysis

Solutions were saturated with high purity N_2, N_2O, O_2 or air as appropriate by flushing with gas for at least 30 min prior to measurements. Phosphate buffer was prepared using NaH_2PO_4 and Na_2HPO_4.

For detection of the primaquine-triplet by laser flash photolysis studies, primaquine diphosphate (0.2 – 0.4 mM) was dissolved in aqueous solutions at pH 7.4 and 9.3 saturated with N_2 or air in the presence and absence of phosphate buffer (10 mM); in N_2-saturated methanol; and in O_2-saturated D_2O at pH 8-9. Primaquine-base ($9.1 \cdot 10^{-5}$ - $3.1 \cdot 10^{-4}$ M) was dissolved in toluene saturated with N_2 or O_2 and in N_2-saturated acetonitrile. Benzophenone dissolved in acetonitrile was used as an actinometer [11].

Primaquine-base was dissolved in O_2-saturated toluene ($^{PQ}Abs_{355}$ = 0.25) for measurements of $^1O_2$ quantum yield by laser flash photolysis. Perinaphthenone in air-saturated toluene ($^{PN}Abs_{355}$ = 0.25) was used as reference [12].

Primaquine-base (0.1-0.3 mM) was dissolved in N_2-saturated acetonitrile in the presence of fenbufen (6 mM) for the study of energy transfer by laser flash photolysis. Fenbufen (6 mM) in acetonitrile was used as reference [13].

Primaquine-base (93 μM) was dissolved in N_2-saturated toluene in the presence of biphenyl (10 mM) for the study of energy transfer by pulse radiolysis. Biphenyl (10 mM) in N_2-saturated toluene was used as reference [14].

Primaquine diphosphate (5.0 μM - 0.2 mM) was dissolved in N_2O-saturated 10 mM phosphate buffer pH 7.4 for investigation of the reaction with the hydroxyl radical OH• by pulse radiolysis. Samples were added 10 mM KBr to verify the formation of PQH^{2+}• and characterize the radical. Samples were saturated with O_2 or N_2O, and mixed to concentration ranges of O_2:N_2O ~ 0:100, 20:80 and 75:25, to evaluate the lifetime of PQH^{2+}• as a function of sample oxygen concentration. The pKa-value and cation radicals of primaquine diphosphate (10 μM and 0.5 mM) formed at various degree of protonation were studied in N_2O-saturated samples at pH pH 2.3 - 11.5. Perchloric acid and NaOH were used for pH adjustments.

The reduction potential under physiological conditions was evaluated by pulse radiolysis of primaquine diphosphate (0.5 mM) in N_2O-saturated 10 mM phosphate buffer pH 7.4 containing 0.1 M KBr, and 5 mM tryptophan (TRPH) or 2 mM tyrosine (TYR).

In order to study the reduction of primaquine by superoxide anions at physiological pH, primaquine diphosphate (0.2 mM) was studied by pulse radiolysis in N_2-saturated 10 mM phosphate buffer pH 7.4 containing 1% t-butanol.

2.2. Laser flash photolysis

The laser flash photolysis experiments were carried out with 15 ns pulses from a J.K. Laser System 2000 Neodynium/YAG oscillator with a Neodynium/glass amplifier. The power dependence measurements used 5 ns pulses from a Continuum Surelight II-10 Nd-YAG laser. Both lasers delivered up to 100 mJ of 355 nm radiation in unfocussed single pulses. The detection system consisted of a water cooled Xe arc lamp and a pulsing unit, high radiance monochromator and quartz optics (Applied Photophysics Ltd.). Optical transmissions (10 mm path length) at various wavelengths selected with the monochromator (bandwidths 2-20 nm) were measured as a function of time before and after the pulse using photoelectric detection. The output of the photomultiplier (Hamamatsu R928) was displayed on a Tektronix TDS 380 digitizing oscilloscope. Data processing was performed on a Dan PC using software developed in house. The sample cell was constructed from Spectrosil quartz and formed part of a flow-through system, introducing fresh solution prior to each laser shot [14, 15]. Benzophenone in acetonitrile was used as a reference.

The luminescence (1270 nm) from excited $^1O_2$ ($^1\Delta_g$) produced by photoexcitation of primaquine in oxygenated solution was detected by a liquid N_2-cooled Germanium Detector / Amplifier (Applied Detector Corporation 403HS) closely coupled to the laser photolysis cell in right-angle geometry [16, 17]. A 1mm thick (20 mm diameter) piece of AR-coated silicon

(II-IV Inc.) was placed between the diode and cell to act as a cut-off filter for light < 1100 nm. The 403HS power supply bias voltage was operated at – 450 V. The amplifier output was AC-coupled to the digitizer.

2.3. Pulse radiolysis

The pulse radiolysis experiments were carried out with a 12 MeV RDL, 3GHz electron linear accelerator with a pulse duration from 0.22 – 2 μs and with a peak current of about 30 mA. It is normally operated at 10 pulses per second but the single pulse mode is achieved by modifying the pulses to the gun [18]. The detection system, similar in principle to the laser flash photolysis apparatus, employed a Kratos monochromator, EMI 9558Q photomultiplier and Tektronix TDS 754C digitizing oscilloscope. The sample cell (Spectrosil quartz) had an optical path length of 25 mm [19]. Radiation doses were measured using the thiocyanate actinometer where the absorption of $(SCN)_2^{\bullet-}$ formed by pulsing an air saturated aqueous solution of 10 mM KSCN is measured taking ε_{500} $(SCN)_2^{\bullet-} = 7.1 \bullet 10^3$ M^{-1} cm^{-1} and $G(SCN)_2^{\bullet-}$ = 2.9 heV^{-1} [20].

2.4. Photochemical degradation

Photodegradation products from primaquine diphosphate (40 μM in D_2O) were formed by irradiation for 5 min. or 15 min. in the Suntest CPS sun simulating exposure unit (1.8 kW Xe-burner; Heraeus GmbH, Hanau, Germany), equipped with a quartz-glass dish with IR reflective coating and a glass filter (transmission 310-800 nm). The energy output was 765 W/m^2, quantified by the XenoCal Sensor equipped with XenoSoft Software (Atlas, Germany) calibrated against filtered radiation (300-800 nm) of a Xe discharge lamp. The samples were irradiated in quartz cells (optical path length 10 mm) during continuous stirring. The degradation was followed by HPLC and UV-vis spectrophotometry. The degradation of primaquine (40 μM) in D_2O was compared to the analogue degradation in water, and the influence of molecular oxygen was investigated by flushing with N_2-gas for 10 min. prior to irradiation.

Photosensitized degradation of primaquine in D_2O was studied using a 900 W Xe arc lamp fitted with a Monochromator *f* 3.4 (Applied Photophysics Ltd., Surrey, England). The samples were irradiated at 455 ± 20 nm up to 60 min. in quartz sample cells (optical path length 10 mm) under continuous stirring at ambient temperature. The adjusted energy at the surface of the container was 72 mV, quantified by a Thermopile voltmeter (200 mV, Applied Photophysics Ltd.) calibrated against a blackbody radiator. The samples contained non-irradiated primaquine diphosphate (40 μM) or primaquine diphosphate (40 μM) irradiated in the Suntest for 5 min. or 15 min. as described above. The samples that were irradiated for 15 min. were added to extra primaquine diphosphate (40 μM) prior to monochromatic irradiation due to extensive decomposition in the Suntest. Additionally, sodium azide (NaN_3 10 mM) was added as a $^1O_2$ quencher prior to monochromatic irradiation in samples that were pre-irradiated for 5 min. Primaquine diphosphate (20 μM in D_2O) was irradiated at 350 ± 20 nm (72 mV) for 0-60 min. as a control of the sensitized reaction. Degradation of primaquine was quantified by use of HPLC and followed by UV-vis spectrophotometry.

2.5. Quantification by HPLC

The concentration of primaquine was quantified by reverse phase HPLC, as previously described [4] (System 2).

2.6. UV-visible absorption spectra

UV-visible absorption spectra (190-700 nm) were recorded by a Shimadzu UV-2401PC UV-vis recording spectrophotometer. The pH of aqueous solutions was adjusted when appropriate by addition of NaOH or $HClO_4$, and controlled by use of a pH-electrode.

2.7. Fluorescence measurements

Fluorescence spectra were measured on a PTI modular Fluorescence System using Felix^{TM} for Windows software. The excitation source was a 75 W xenon lamp. The monochromators were Model 101 (*f*/4 0.2 m Czerny-Turner configuration).

Fluorescence lifetimes (Ex. 337 nm; Em. 527 nm) of primaquine diphosphate in water (Abs_{337} = 0.31), primaquine diphosphate in D_2O (Abs_{337} = 0.39) and primaquine diphosphate in D_2O (40 μM) irradiated in the Suntest for 20 min. (resulting in Abs_{337} = 0.08) were measured. Riboflavin in purified water (Abs_{337} < 0.1) was used as reference (Ex. 337 nm; Em. 538 nm).

Fluorescence quantum yields (Φ_f) of primaquine diphosphate in water and in D_2O (Ex. 350 nm, Em. 370-700 nm; $Abs_{350} \leq 0.115$) and of primaquine diphosphate in D_2O (40 μM) irradiated in the Suntest for 20 min. (Ex. 455 nm, Em. 470-700 nm, Abs_{455} = 0.086) were calculated. Fluorescence quantum yields were obtained by using quinine sulphate dissolved in 0.05 M H_2SO_4 as reference (Φ_f = 0.55) [21].

The formation of $^1O_2$ sensitized by primaquine diphosphate in D_2O (Ex. 455 nm) and photoproducts of primaquine diphosphate in D_2O (40 μM) irradiated in the Suntest for 20 min. was investigated by direct detection of $^1O_2$ luminescence at 1270 nm in a steady state mode. The detector was an EQ-817 Germanian Detector System operated under liquid N_2 operations. Rose Bengal in D_2O was used as reference (Ex. 548 nm).

3. Results and discussion

Primaquine is an aminoquinoline with pK_a values = 3.2 (quinoline N) and 10.4 (amine group) [22]. The pK_a value of the aniline N is very low (< -1), likely due to delocalization of the lone-pair electrons of this group into the aromatic ring. At physiological pH primaquine exists as the monocation PQH^+ (Scheme 1). A summary of the photophysical properties and reaction rates of primaquine transients are presented in Tab.1.

3.1. Studies of the excited state of primaquine by laser flash photolysis and pulse radiolysis

Following 355 nm laser flash photolysis of a nitrogen saturated aqueous solution of primaquine (0.1 mM), transient absorption bands centred at 410 and 720 nm were observed at the end of pulse, the 720 nm band decayed with a first order rate of $2 \bullet 10^6$ s^{-1} while that at 410 nm was found to be long lived and decayed by a second order process (Fig. 1). Moreover, the long wavelength absorption band was quenched within the pulse in the presence of nitrous oxide. On the basis of the absorption spectrum and the reaction with nitrous oxide, the long wavelength absorption band is assigned to the hydrated electron formed presumably by reaction (1):

$$^1PQH^{+}* \rightarrow PQH^{2+}\bullet + e^-_{aq} \qquad (1)$$

On saturating the solution with oxygen, it was found that the long wavelength band rapidly decayed ($\tau < 0.2$ μs) while that centred at 410 nm showed only a very slight increase in the decay rate. On the basis of the above we have assigned this band (410 nm) to the cation radical of primaquine. This assignment was confirmed by pulse radiolysis (see section 3.2) and is in agreement with the previous work by Viola et al. [23]. Figure 1B shows the difference absorption spectrum of the long lived species in air saturated aqueous solution of primaquine.The difference between the 5 μs spectrum (Fig. 1A) and 1B (in 370-600 nm wavelength region) is explicable in terms of the absorption due to the anion radical of primaquine formed by the reaction of the photoejected hydrated electron with primaquine in 1A where as the electron is quenched by oxygen in 1B. Primaquine anion radical is shown to have a broad absorption band with maximum at 490 nm [24]. Furthermore, this shows that the superoxide anion radical formed by the reaction of hydrated electron with oxygen does not reduce primaquine as will be shown in section 3.3 by pulse radiolysis.

In order to determine whether the photoionisation is monophotonic or not, we determined the effect of laser intensity on the transient formation. It was found that both 720 and 410 nm absorption increased non-linearly with the laser intensity (see inset in Fig. 1B), indicating that the photoionisation is not a monophotonic process. From the log plot (inset (b) Fig. 1B) we obtained a value of 1.6 instead of 2 due to a biphotonic process. This may indicate a mixed mono and bi- photonic process; we have failed to observe significant transient absorbance at either 700 or 410 nm under experimental conditions that otherwise produce normal one-photon excitation in past experiments. Stevenson et al. [25] note that the observed two photon power dependence is less with an experimental setup in which the laser and analyzing light beams are perpendicular (as in our experiment) than in an arrangement with collinear beams. In addition it is noted that at the powers and concentrations used in our experiments, saturation of absorption is likely. These factors may explain why we observe power dependencies of less than 2 in experiments such as those illustrated in Figure 1. From the above experiments no absorption due to the triplet state could be detected in the UV/ vis region. It may be that the triplet state is too short lived and/or its absorption coefficient is too small for our detection system to detect. Various media were used for a thorough investigation but no transient attributable to the primaquine triplet was detected.

The luminescence at 1270 nm from excited $^1O_2$ ($^1\Delta_g$) sensitized by photoexcitation (355 nm) of primaquine ($^{PQ}Abs_{355} = 0.25$) in O_2-saturated toluene was then measured as an indirect detection of the primaquine triplet (Fig. 2). Perinaphthenone in air-saturated toluene ($^{PN}Abs_{355} = 0.25$) was used as reference. For any given laser energy, the number of photons absorbed by the two solutions will be the same since both have the same absorbance at the wavelength of excitation (355 nm). The quantum yield of $^1O_2$ formation sensitized by primaquine ($^{PQ}\Phi_\Delta$) is 0.025, as calculated from

$$^{PQ}\Phi_\Delta / {}^{PN}\Phi_\Delta = {}^{PQ}I_e / {}^{PN}I_e \qquad \text{Eq. 1}$$

where $^{PN}\Phi_\Delta$ is taken as 0.98 [12]; $^{PQ}I_e$ and $^{PN}I_e$ are initial emission intensities for primaquine and perinaphthenone, respectively, at any given laser intensity and are obtained by the slopes of the lines in Fig. 2 ($^{PQ}I_e = 1.06$, $R^2 = 0.977$; $^{PN}I_e = 42.25$, $R^2 = 0.999$). This is in agreement with a value of 0.01 in air saturated benzene [26]. However, Motten et al. [27] did not detect any significant $^1O_2$ formation induced by primaquine in D_2O at pD 7 ($^{PQ}\Phi_\Delta < 0.005$).

At pulse energy of 9.50 mJ the initial intensity of the emission is reduced from 10.2 mV to 5.4 mV by shaking the O_2-saturated sample of primaquine in toluene with air prior to excitation. Moreover, ${}^{PQ}I_e$ is doubled (factor 2.2) on increasing the primaquine concentration from $9.1 \bullet 10^{-5}$ M to $6.4 \bullet 10^{-4}$ M. These observations verify the formation of ${}^1O_2$ during photoexcitation of primaquine. It seems likely that ${}^1O_2$ is formed by energy transfer from the short lived primaquine triplet (${}^3PQ^*$) in accordance with reaction (2):

$$ {}^3PQ^* + O_2 \rightarrow {}^1O_2 + PQ \qquad (2) $$

Sensitization experiments were performed to further confirm the existence of the primaquine triplet. Energy transfer was studied by laser flash photolysis at 355 nm of primaquine (0.3 mM) in N_2-saturated acetonitrile in the presence of fenbufen (6 mM). Fenbufen in acetonitrile shows transient absorption centred at 420 nm which is assigned to its triplet state [13]. The lifetime of the fenbufen triplet is 10±0.5 μs, calculated by the first order decay of the transient at 420 nm ($k_{ref} = 9.9 \pm 0.4 \bullet 10^4$ s^{-1}, n = 2). Primaquine reduces this lifetime by about 90%. But no absorption due to the primaquine triplet could be detected. On reducing the concentration of primaquine to 0.1 and 0.2 mM, the fenbufen triplet was quenched at the rate of $k_{obs} = 4 \bullet 10^5$ and $7 \bullet 10^5$ s^{-1} respectively. From these data the bimolecular rate constant for quenching of fenbufen triplet by primaquine was calculated to be $k_q = 3 \bullet 10^9$ M^{-1} s^{-1}. The quenching rate constant is calculated from

$$ k_q\ (M^{-1}s^{-1}) = k_{obs}\ (s^{-1}) - k_{ref}\ (s^{-1}) \,/\, [\text{primaquine}]\ (M) \qquad \text{Eq. 2} $$

Thus primaquine is an efficient quencher of the fenbufen triplet. It seems likely that, as the fenbufen triplet decays, the primaquine triplet is formed in accordance with reaction (3):

$$ {}^3FEN^* + PQ \rightarrow {}^3PQ^* + FEN \qquad (3) $$

Sensitization of primaquine (93 μM) in N_2-saturated toluene was further investigated by pulse radiolysis in the presence of biphenyl (10 mM). The biphenyl triplet is formed by pulse radiolysis during energy transfer from excited toluene [14]. The first order decay of the biphenyl triplet in toluene at 360 nm ($k_{ref} = 2.2 \bullet 10^4$ s^{-1}; n = 4; RSD = 20 %) is increased in the presence of primaquine ($k_{obs} = 3.8 \bullet 10^5$ s^{-1}; n = 3; RSD = 10 %). Primaquine is an efficient quencher of the biphenyl triplet with a bimolecular quenching rate constant $k_q = 3.9 \bullet 10^9$ $M^{-1}s^{-1}$, as calculated by Eq. 2.

As the biphenyl triplet decays, it seems likely that the primaquine triplet is formed in accordance with reaction (4):

$$ {}^3BP^* + PQ \rightarrow {}^3PQ^* + BP \qquad (4) $$

Existence of a short lived primaquine triplet (< 50 ns) is likely, based on the sensitization experiments, formation of singlet oxygen and on the effect of oxygen concentration on the yield of singlet oxygen. Failure to observe the triplet-triplet absorption spectrum of primaquine under our conditions may be due to either the short lifetime and/or very small molar absorption coefficient in the wavelength region analysed.

3.2. Identification and characterizations of the cation radicals of primaquine by pulse radiolysis

As seen in the previous section primaquine is photoionised in a biphotonic process to form the cation radical. We have used the techniques of pulse radiolysis to determine and characterise the cation radical.

The reaction of the hydroxyl radical OH• with monoprotonated primaquine (PQH^+) was studied by pulse radiolysis of an N_2O-saturated solution of primaquine (0.2 mM) in 10 mM phosphate buffer pH 7.4. Under these conditions the initially produced hydrated electrons are scavenged by N_2O, leaving only OH• as the reactive radical [14]. Fig. 3 shows the transient difference absorption spectra observed 7 μs and 191 μs after the pulse. Under these conditions a broad band centred at 400 nm and a very broad band in the visible region are observed at the end of OH• reaction (7 μs). However at 191 μs the band centred at 400 nm narrows and shifts to 410 nm. OH• radicals are known to react with organic compounds by addition to double bonds, hydrogen abstraction, and electron abstraction by formation of cation radicals as in the case of the antimalarial mefloquine [17]. The bimolecular quenching rate constant of OH• by primaquine $k_q = 6.6 \bullet 10^9\ M^{-1}s^{-1}$, calculated as the slope of the line ($R^2 = 0.957$) when the pseudo first order rate for the growth of transient absorption at 410 nm was plotted as a function of primaquine concentration at 5.0-10.0 μM ($n \leq 3$). The rate of reaction is in the order of magnitude expected for the addition of OH• radicals to, or abstraction of an electron / H-atom from, aromatic compounds [28]. Hence, the spectrum shown in Fig.3 could be either the primaquine monoprotonated cation radical and/or other products, such as an OH• - addition product, as illustrated in reaction (5):

$$OH\bullet + PQH^+ \rightarrow PQH^{2+}\bullet + OH^- \text{ or } PQ(OH)H^+\bullet \qquad (5)$$

The formation of $PQH^{2+}\bullet$ was confirmed and the monoprotonated cation radical further characterized by pulse radiolysis of primaquine (0.2 mM) in N_2O-saturated 10 mM phosphate buffer pH 7.4 containing 10 mM KBr. The one electron oxidizing radical $Br_2\bullet^-$ is produced when N_2O-saturated aqueous solutions of KBr are subject to pulse radiolysis [14]. Fig. 4 shows the transient difference absorption spectra observed at 5 μs and 51 μs after the pulse. The transient absorption maxima at 410 nm and 560 nm are due to formation of $PQH^{2+}\bullet$ during oxidation of primaquine by the $Br_2\bullet^-$ radicals at neutral pH, in accordance with reaction (6):

$$Br_2\bullet^- + PQH^+ \rightarrow PQH^{2+}\bullet + 2Br^- \qquad (6)$$

Both 410 and 560nm absorption decayed by a second order process with $k_2 = 7.4 \pm 0.6 \bullet 10^8\ M^{-1}s^{-1}$, hence both bands are due to the same species, $PQH^{2+}\bullet$. It is clear, from a comparison of the spectra in Figs. 3 and 4, that less than half of OH• reaction with primaquine results in the formation of $PQH^{2+}\bullet$.

$Br_2\bullet^-$ shows characteristic transient absorption at 360 nm. By plotting the pseudo first order rate for the decay of transient absorption at this wavelength as a function of primaquine concentration at zero, $1.0 \bullet 10^{-5}$ M, $2.5 \bullet 10^{-5}$ M and $5.0 \bullet 10^{-5}$ M ($n \leq 3$), the bimolecular quenching rate constant was calculated as the slope of the line ($R^2 = 0.998$); $k_q = 4.7 \bullet 10^9\ M^{-1}s^{-1}$.

Primaquine forms oxygenated photoproducts in a complex degradation pattern which is highly dependent on the oxygen content of the medium [2, 4]. Thus the lifetime of $PQH^{2+}\bullet$ as a function of oxygen concentration was studied by pulse radiolysis of primaquine (0.2 mM) in 10 mM phosphate buffer pH 7.4 containing 10 mM KBr. Since $PQH^{2+}\bullet$ decayed essentially by

a second order process, the dose was kept as low as possible (~ 2.5 Gy) and adjusted to ensure the initial concentration of PQH^{2+}• remained the same for this set of experiments. Samples were saturated with O_2 or N_2O, and mixed to concentration ranges of O_2:N_2O ~ 0:100, 20:80 and 75:25. The lifetime of PQH^{2+}• was calculated by the pseudo first order decay of the transient absorption at 410 nm ($n \leq 4$; $R^2 = 0.980$) as a function of per cent oxygen content (Fig. 5). O_2 will influence degradation of the drug by direct reactions with PQH^{2+}• according to reaction (7):

$$PQH^{2+}\bullet + O_2 \rightarrow PQox \qquad (7)$$

where PQox is oxidized degradation product(s).

The bimolecular quenching rate constant of PQH^{2+}• by O_2, $k_q = 1.0 \bullet 10^6$ $M^{-1}s^{-1}$, was calculated from the slope of the plot of the pseudo first order rate of the transient decay at 410 nm (PQH^{2+}•) vs. the molar oxygen concentration ($n \leq 4$; $R^2 = 0.962$). The oxygen content in water under pure oxygen at 25 ºC was taken as $1.29 \bullet 10^{-3}$ M, according to Connors et al. [29].

The reduction potential of primaquine under physiological conditions was further evaluated by pulse radiolysis of primaquine (0.5 mM) in N_2O-saturated 10 mM phosphate buffer pH 7.4 containing 5 mM tryptophan (TRP) and 0.1 M KBr. Fig. 6 shows the transient difference absorption spectra observed at 7 μs, 27 μs and 69 μs after the pulse. The maximum at 520 nm is due to formation of the deprotonated cation radical of tryptophan by reaction (8a & 8b) in the presence of $Br_2\bullet^-$:

$$Br_2\bullet^- + TRPH \rightarrow TRPH^+\bullet + 2Br^- \qquad (8a)$$

$$TRPH^+\bullet \rightarrow TRP\bullet + H^+ \qquad (8b)$$

The corresponding decays of TRP• (520 nm) and increase in PQH^{2+}• (410 nm) shows that primaquine is a quencher of TRP• according to reaction (9):

$$TRP\bullet + PQH^+ + H^+ \rightarrow PQH^{2+}\bullet + TRPH \qquad (9)$$

The bimolecular quenching rate constant of TRP• by PQH^+, $k_q = 2.5 \pm 0.3 \bullet 10^7$ $M^{-1}s^{-1}$, was determined from the pseudo first order rate constant for the decay of TRP• absorption at 520 nm in the absence of primaquine ($k_{ref} = 1.6 \pm 0.6 \bullet 10^3$ s^{-1}, n = 4) and in the presence of 0.5 mM primaquine ($k_{obs} = 1.4 \pm 0.1 \bullet 10^4$ s^{-1}, n = 2), and calculated according to Eq. 2. The pseudo first order rate constant for the decay of TRP• when quenched by PQH^+ ($k_{obs} = 1.4 \pm 0.1 \bullet 10^4$ s^{-1}, n = 2) as measured at 520 nm corresponds well with the rate constant for the increase of PQH^{2+}• measured at 410 nm ($k_1 = 1.6 \bullet 10^4$ s^{-1}, n = 1). This is expected according to reaction (9), and verifies that primaquine (PQH^+) acts as a quencher of TRP•. The reduction potential E° (PQH^{2+}• / PQH^+) at physiological pH is thus lower than +1015 mV, which is the value for E° (TRP• / TRPH) at neutral pH [30].

The reduction potential of PQH^{2+}• / PQH^+ was also evaluated by pulse radiolysis of primaquine (0.5 mM) in N_2O-saturated 10 mM phosphate buffer pH 7.4 containing 2 mM tyrosine (TYR) and 0.1 M KBr. Tyrosine will not form radicals in reactions with $Br_2\bullet^-$ at these concentrations due to a much faster reaction rate between PQH^+ and $Br_2\bullet^-$. The apparent first order decay of PQH^{2+}• absorption detected at 410 nm ($k_1 = 3.0 \pm 0.7 \bullet 10^3$ s^{-1}, n = 3) is not changed in the presence of tyrosine ($k_1 = 3.1 \pm 0.7 \bullet 10^3$ s^{-1}, n = 2). The tyrosine radical, detected

in a separate experiment at 410 nm in the absence of primaquine, has a faster decay (k_1 = $1.8 \cdot 10^4$ s^{-1}, n = 1). The reduction potential E° ($PQH^{2+}\bullet$ / PQH^+) is thus likely to be even lower than +930 mV, which is the reduction potential E° (TYR• / TYR) at neutral pH [30]. However, it can not be excluded that that the quenching of $PQH^{2+}\bullet$ by tyrosine is not observed due to a low reaction rate.

The long-lived nature of the 390-400 nm / 550-560 nm absorbing species formed in the reaction between PQH^+ and OH• / $Br_2\bullet^-$, its relatively slow reactivity with oxygen (k_q = $1.0 \cdot 10^6$ $M^{-1}s^{-1}$) and the fact that it is formed from the one electron oxidizing radical $Br_2\bullet^-$ leads to the conclusion that this is the monoprotonated cation radical of primaquine ($PQH^{2+}\bullet$). As $PQH^{2+}\bullet$ is formed photochemically by a two-photon process, direct photoionisation by solar or room light exposure is not likely. However, the radical cation might be formed by photosensitized reactions of types I or II, as illustrated in Scheme 2. The relatively low reduction potential of primaquine at physiological pH (E° ($PQH^{2+}\bullet$ / PQH^+) < +1015 mV) combined with the long lifetime makes primaquine subject to oxidative degradation by formation of the monoprotonated cation radical. Reaction (7) is previously suggested as an important initial process in the photochemical degradation of primaquine [4]. Oxidation of the monoprotonated cation radical by molecular oxygen will make an important contribution to degradation under aerobic conditions. Previous studies also indicate that OH• is formed during photochemical degradation of primaquine (e.g. by degradation products). Formation of the OH• radical will increase the decomposition of primaquine in aqueous solution, as primaquine is oxidized by OH• at a high rate ($k_q = 6.6 \cdot 10^9$ $M^{-1}s^{-1}$).

Physicochemical properties and photoreactivity of primaquine is highly influenced by the protonation/deprotonation of the drug (Scheme 1). Hence, the formation of cation radicals of primaquine at various degree of protonation was studied by pulse radiolysis of primaquine (0.5 mM) in N_2O-saturated aqueous solutions containing 10 mM KBr at pH 2.3 (PQH_2^{2+}), pH 5.6 (PQH^+) and pH 10.1 (PQ). Fig. 7 shows the corrected (for ground state bleeching) transient absorption spectra that were observed immediately after the pulse (6 μs at pH 2.3; 3 μs at pH 5.6 and 8 μs at pH 10.1, respectively). The transients at pH 2.3 and 5.6 have absorption maxima at 400-410 nm and 550-570 nm, i.e. identical with the spectrum detected at pH 7.4 (Fig. 4). The transient at pH 10.1 shows a different absorption spectrum, with a red shift of the first absorption maximum to 650 nm, a new (small) maximum at 470 nm and a blue shift to 385 nm (Fig. 7). The primaquine cation radical is most likely centred at the quinoline nitrogen. The proton that is attached to this functional group at acidic pH will thus be repulsed during the oxidation reaction with the $Br_2\bullet^-$ radicals. The cation radicals formed at pH 2.3; 5.6 and pH 7.4 will then be identical, which is verified by equal second order rate constants for the transient decay ($k_2 = 6.0\text{-}7.4 \cdot 10^8$ $M^{-1}s^{-1}$) and illustrated in reactions (6) and (10). The transient absorption spectrum shows a different profile at alkaline pH, due to the shorter lived ($k_2 = 1.0 \cdot 10^9$ $M^{-1}s^{-1}$) non-protonated radical that is formed in accordance with reaction (11):

$$Br_2\bullet^- + PQH_2^{2+} \rightarrow PQH^{2+}\bullet + H^+ + 2Br^- \qquad (10)$$

$$Br_2\bullet^- + PQ \rightarrow PQ^+\bullet + 2Br^- \qquad (11)$$

The reactions are illustrated in Scheme 1. The intramolecular repulsion between charged groups in $PQH^{2+}\bullet$ will favour intermolecular interactions with the medium, while an intramolecular interaction between the quinoline radical centre and amine group in $PQ^+\bullet$ is likely to shield part of the transient from the surroundings. This can explain the differences in

the transient absorption spectra as well as the longer lifetime of PQH^{2+}• compared with that of PQ^{+}• It is interesting to compare these observations with absorption properties in the ground state, which are similar at alkaline and neutral pH (maximum at 350 nm, spectrum extended until 430 nm). Acidification and formation of the ground state primaquine dication leads to formation of two absorption maxima at 330 and 420 nm, respectively, and extension of the spectrum towards 500 nm. The first absorption band of primaquine in the ground state represents the transition of the lone-pair electrons (n-π* transition) at the quinoline nitrogen. Intramolecular bonding is favoured in both alkaline and neutral media, leading to similar absorption properties. Intramolecular charge repulsion and extended interaction with the medium change the absorption properties at acidic pH [5].

The bimolecular quenching rate constants of Br_2•$^-$ by PQH_2^{2+} (pH 2.4) and PQ (pH 10.8) are $k_q = 2.7\pm0.2\bullet10^9$ $M^{-1}s^{-1}$ and $k_q = 4.2\pm0.1\bullet10^9$ $M^{-1}s^{-1}$, respectively, as determined by the pseudo first order rate for the decay of transient absorption at 360 nm (Br_2•$^-$) detected in an N_2O-saturated aqueous medium containing 10 mM KBr ($k_{ref} = 4.5\pm0.2\bullet10^3$ s^{-1}at pH 2.4, n = 3; $k_{ref} = 4.6\pm0.4\bullet10^3$ s^{-1}at pH 10.8, n = 2) and in the presence of 10 μM primaquine ($k_{obs} = 3.1\pm 0.1\bullet10^4$ s^{-1}at pH 2.4, n = 2; $k_{obs} = 4.7\pm 0.05\bullet10^4$ s^{-1}at pH 10.8, n = 3). The quenching rates are calculated according to Eq. 2. The quenching rate constant at pH 7.4 (PQH^{+}) is previously determined to be $k_q = 4.7\bullet10^9$ $M^{-1}s^{-1}$. Primaquine as a monocation (neutral pH) and non-ion (alkaline conditions) thus undergo one electron oxidization by Br_2•$^-$ radical at diffusion controlled rates, while the dication (acidic medium) is oxidized at about half the rate. Protonation of the quinoline N and binding of the reactive n-electrons at low pH can explain the reduced reaction rate of the dication compared to the two other forms (Scheme 1).

The molar absorption coefficient (Є) of primaquine transients as a function of pH were calculated from the transient absorption maximum (A_{max}) detected immediately after the laser pulse (3-12 μs), the optical path length (2.5 cm) and estimated concentration of transient assuming the yield of e_{aq}^- to be 2.7 μM $kRad^{-1}$ and 100% efficiency of the radical reactions:

$$Є\ (M^{-1}\ cm^{-1}) = A_{max} / [\text{PQ-transient}]\ M \bullet 2.5\ cm \qquad \text{Eq. 3}$$

The monoprotonated cation radical (PQH^{2+}•) has the same molar absorption coefficient in aqueous media at pH 5.6 and 7.4 ($Є_{400}$ = 7300 M^{-1} cm^{-1}; $Є_{550}$ = 1700-1800 M^{-1} cm^{-1}). In an acidified medium (pH 2.3) the calculated molar absorption coefficients are only slightly changed ($Є_{410}$ = 6500 M^{-1} cm^{-1}; $Є_{570}$ = 2000 M^{-1} cm^{-1}).

The pK_a-value of the cation radicals (PQH^{2+}•/ PQ^{+}•) was evaluated by pulse radiolysis of primaquine (10 μM) in N_2O-saturated aqueous solutions containing 10 mM KBr at pH 2.4; 7.1; 8.1; 9.5; 10.8 and 11.5. The plot of transient absorbance at 560 nm (PQH^{2+}•) as a function of pH is presented in Fig. 8. The steep decline in absorbance between pH 7 and 8 indicates that PQH^{2+}•/ PQ^{+}• has a pK_a-value in this range, which is a significant change from the ground state (pKa = 10.4). The corresponding increase in transient absorption at 640 nm (PQ^{+}•) with an increase in pH did not demonstrate any characteristic behaviour that could be used in the calculations.

3.3. Studies of the scavenging reaction between primaquine and superoxide by pulse radiolysis

In order to study the reduction of primaquine by superoxide anions at physiological pH, the radical anion of primaquine (protonated / deprotonated) was identified by pulse radiolysis of

primaquine (0.2 mM) in N_2-saturated 10 mM phosphate buffer pH 7.4 containing 1% t-butanol. The initially produced OH• and H• radicals react rapidly with t-butanol producing relatively unreactive t-butanol radicals [14]. The hydrated electron (e_{aq}^-) is unaffected by the presence of t-butanol and is free to react with primaquine according to reaction (12):

$$e_{aq}^- + PQH^+ \rightarrow PQH\bullet \leftrightarrows PQ\bullet^- + H^+ \quad (12)$$

According to Bisby [24] the unprotonated 8-aminoquinoline ring of primaquine appears to be the site for reduction by e_{aq}^- at a diffusion controlled rate ($k_q = 2.47\pm0.1\bullet10^{10}$ $M^{-1}s^{-1}$ at pH 7.3). The anion radical is supposed to be deprotonated (PQ•-) at neutral pH based on measured electron transfer reaction rates. The strongly reducing primaquine anion radical (E°, PQH^+/ PQ•- < -1022 mV) is efficiently quenched by molecular oxygen ($k_q = 2.31\pm0.25\bullet10^9$ $M^{-1}s^{-1}$ at pH 6.9), likely by one-electron transfer by formation of superoxide anions (E°, $O_2/O_2\bullet^-$ = -160 mV). According to these observations, the reverse reaction is not very likely.

The molar absorption coefficient of the anion radical was calculated by Eq. 3 at the absorption maximum at 350 nm (ϵ = 4700 M^{-1} cm^{-1}) and 490 nm (ϵ = 2100 M^{-1} cm^{-1}) (spectrum not shown). The hydrated electron has an absorption maximum at 720 nm and will not interfere with the spectrum [14]. The lifetime of PQH• / PQ•- in an anaerobic medium is in the milliseconds.

One-electron transfer from the superoxide radical anion ($O_2\bullet^-$) to primaquine at physiological pH (PQH^+) was subsequently studied by pulse radiolysis of primaquine (0.1 mM) in O_2-saturated 10 mM phosphate buffer pH 7.4 containing 0.1 M sodium formate to increase the yield of $O_2\bullet^-$. Pulse radiolysis of the sample produces superoxide radicals by reactions (13-15) [14]:

$$e_{aq}^- + O_2 \rightarrow O_2\bullet^- \quad (13)$$

$$OH\bullet + HCO_2^- \rightarrow CO_2\bullet^- + H_2O \quad (14)$$

$$CO_2\bullet^- + O_2 \rightarrow O_2\bullet^- + CO_2 \quad (15)$$

As expected, no reaction between primaquine and $O_2\bullet^-$ could be detected, as investigated at the absorption maximum of the primaquine anion radical (PQH•/ PQ•-) at 480 nm, illustrated by reaction (16):

$$O_2\bullet^- + PQH^+ \not\rightarrow PQH\bullet + O_2 \leftrightarrows PQ\bullet^- + H^+ + O_2 \quad (16)$$

The result is verified by the fact that no bleaching (detected as negative absorption) is observed by primaquine in the ground state at 360 nm (data not shown). These findings are in accordance with previous scavenging studies, where superoxide dismutase did not reduce photodegradation of primaquine although $O_2\bullet^-$ is formed in the reactions [3, 4]. Hence, primaquine will not be decomposed by self-sensitization by $O_2\bullet^-$ in the photochemical degradation process.

3.4. Photosensitized degradation of primaquine

The photochemical degradation of primaquine in the Suntest is significantly accelerated in deuterium oxide (D_2O) compared to purified water, as 10(±3) % (n = 2) was decomposed

after only 5 min. irradiation in D_2O while the drug had to be irradiated for 60 min. in water to obtain 13(±2) % (n = 2) decomposition. Reduction of the oxygen content by flushing with N_2-gas prior to irradiation in D_2O reduces the degradation from 86(±1) % (n = 2) to 45(±10) % (n = 3) after 15 min. exposure. The influence of oxygen concentration and the increased degradation in D_2O indicate that $^1O_2$ is involved in the reaction, which is likely since primaquine generates some $^1O_2$ during laser flash photolysis ($^{PQ}\Phi_\Delta = 0.025$) and the drug is a quencher of $^1O_2$ at moderate rate ($k_q = 2.6 \bullet 10^8$ M^{-1} s^{-1}) [27]. The first absorption band of ground state primaquine at 350 nm is decreased during photodegradation parallel to growth of a new maximum at 455 nm (i.e. outside the original absorption range). Irradiation at 455 nm was thus selected for investigation of a photosensitized degradation. Based on quenching studies we have previously suggested that initially formed photodegradation products or impurities of the raw material are sources of $^1O_2$ in the photochemical reactions [4].

The photosensitized degradation of primaquine by monochromatic irradiation at 455 nm is illustrated in Fig. 9. The reactions can be expressed by apparent zero order degradation kinetics and the corresponding degradation rates (k_{obs}) are given as relative values obtained under the selected experimental conditions. The results emphasize the possibility that one or more impurities in the raw material sensitize photodegradation of the drug ($k_{obs} = 3.8 \bullet 10^{-9}$ M s^{-1}, $R^2 = 0.947$). Samples spiked with photochemical degradation products prior to exposure at 455 nm show increased degradation. Degradation products that are formed initially seem to be the most important, as the photosensitized degradation after irradiation in the Suntest for 5 min. ($k_{obs} = 1.2 \bullet 10^{-8}$ M s^{-1}, $R^2 = 0.999$) and 15 min. ($k_{obs} = 1.5 \bullet 10^{-8}$ M s^{-1}, $R^2 = 0.985$) are in the same range. The $^1O_2$ quencher sodium azide (NaN_3) prevents the reaction at the concentration added (10 mM). No significant degradation was observed in 5 min. irradiated samples during monochromatic irradiation at 455 nm for 15 min. after addition of the quencher, or in non-decomposed samples that were irradiated monochromatically for 30 or 60 min. in the presence of the quencher. These results support the hypothesis of $^1O_2$ mediated photosensitized degradation of primaquine by impurities of the raw material and photodegradation product(s), although we were not able to detect $^1O_2$ luminescence at 1270 nm by excitation at 455 nm. Low concentration(s) of the sensitizer(s) combined with low $^1O_2$-quantum yield(s) may be the reason.

The fluorescence quantum yield for the photodegradation product(s) of primaquine in D_2O appears to be low ($\Phi_F \sim 6 \bullet 10^{-4}$), and the fluorescence lifetime is short ($\tau_F < 0.5$ ns). The fluorescence quantum yield of primaquine in water is low ($\Phi_F \sim 9 \bullet 10^{-4}$) and is only slightly increased in D_2O ($\Phi_F \sim 2 \bullet 10^{-3}$). The fluorescence lifetime of primaquine is short ($\tau_F < 0.5$ ns) in water and in D_2O.

Addition of NaN_3 partly retards photodegradation of primaquine in D_2O when irradiated in the Monochromator at 350 nm, as percentage degradation is reduced from 10(±1) % (n = 2) to 5(±2) % (n = 4) after 30 min. irradiation in the presence of the quencher. Hence free radical reactions also contribute to photodegradation of primaquine, as postulated earlier by a series of scavenging experiments [3, 4, 7]. Formation and reactivity of the monoprotonated cation radical is expected to play an essential part in the initial photochemical degradation of primaquine at physiological pH, as illustrated in Scheme 2. Formation of new photosensitizers (PQox) during drug photodegradation will accelerate the initially slow degradation rate with time, as previously observed [4].

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Figure and table legends

Figure 1
Transient difference absorption spectra from laser flash photolysis (355 nm) of primaquine in aqueous solution at pH 7.2 (laser energy ~50 mJ/pulse).
A: Primaquine (0.1 mM) in N_2-saturated solution 0.5 μs (■) and 5 μs (□) after the pulse; Inset – transient decays at 410 nm (a) and 720 nm (b). B: Primaquine (0.3 mM) in air-saturated solution, 5 μs after the pulse: Insets – power dependence of transient absorbance extrapolated to zero time at 700 nm (■) for N_2 saturated 0.1 mM primaquine solution and 410 nm (□) for air saturated 0.3 mM primaquine solution as a) linear, and b) log-log plots.

Figure 2
A plot of initial emission intensity (mV) at 1270 nm due to $^1O_2$ as a function of laser energy (mJ) at 355 nm for O_2-saturated toluene solution of primaquine-base (●) and air-saturated toluene solution of perinaphthenone (□). Both solutions had the same absorbance of 0.25 at 355 nm.

Figure 3
Transient difference absorption spectra observed at 7 μs (□) and 191 μs (●) after pulse radiolysis of primaquine (0.2 mM) in N_2O-saturated 10 mM phosphate buffer pH 7.4 (spectra normalised to a Dose of 8 Gy). Inset shows the changes in transient absorption at 400 nm (PQH^{2+}•).

Figure 4
Transient difference absorption spectra observed at 5 μs (□) and 51 μs (●) after pulse radiolysis of primaquine (0.2 mM) in N_2O-saturated 10 mM phosphate buffer pH 7.4 containing 10 mM KBr (spectra normalised to a Dose of 8 Gy). Inset shows the changes in transient absorption at 410 nm (PQH^{2+}•).

Figure 5
Lifetime of PQH^{2+}• (ms) as a function of sample oxygen concentration (% saturation) studied by pulse radiolysis of primaquine (0.2 mM) in O_2/N_2O-saturated 10 mM phosphate buffer pH 7.4 containing 10 mM KBr, detected as transient decay at 410 nm ($R^2 = 0.980$). Inset shows the changes in transient absorption at 410 nm under 75 % O_2-saturation.

Figure 6
Transient difference absorption spectra observed at 7 μs (□), 27 μs (●) and 69 μs (Δ) after pulse radiolysis of primaquine (0.5 mM) in the presence of tryptophan (5 mM) in N_2O-saturated 10 mM phosphate buffer pH 7.4 containing 0.1 M KBr. Insets show the changes in transient absorption at 520 nm and 410 nm, respectively.

Figure 7
Corrected (for ground state bleaching) transient absorption spectra at various degree of primaquine ionization, studied by pulse radiolysis of primaquine (0.5 mM) in N_2O-saturated aqueous solutions containing 10 mM KBr: pH 2.3 detected 6 μs after the pulse (●), pH 5.6 detected 3 μs after the pulse (□), pH 10.1 detected 8 μs after the pulse (Δ).

Figure 8
Transient absorbance extrapolated to the pulse at 560 nm as a function of pH, observed by pulse radiolysis of primaquine (10 μM) in N_2O-saturated aqueous solutions containing 10 mM KBr.

Figure 9
Photosensitized degradation of primaquine in D_2O by irradiation at 455 (± 20) nm (72 mV). The samples contained primaquine diphosphate (○); primaquine diphosphate ($4 \cdot 10^{-5}$ M) pre-irradiated in the Suntest CPS (Atlas) for 5 min. (■) or primaquine diphosphate ($4 \cdot 10^{-5}$ M) pre-irradiated in the Suntest CPS (Atlas) for 15 min. and supplied with an additional amount of drug prior to monochromatic irradiation (▲). Each point is the mean of 3 measurements ($R^2 \geq 0.946$).

Scheme 1
Postulated formation of primaquine (PQ) cation radicals at various degree of drug protonation.

Scheme 2
Postulated mechanisms for the photosensitized degradation of primaquine (PQH^+) in a neutral aqueous medium.
Sens = photosensitizing impurities in the raw material
PQox = oxidized photodegradation products of primaquine

Table 1
A summary of the photophysical properties and reaction rates of primaquine transients

Transient / reaction	Solvent / pH	Є ($M^{-1}cm^{-1}$) (λ_{max}, nm)	k_2 ($M^{-1}s^{-1}$) (λ, nm)	$^{PQ}\Phi_{\Delta}$	k_q ($M^{-1}s^{-1}$)
PQ^{+}•	Aqueous pH 10.1	5500 (385)			
		800 (470)			
		1600 (650)	$1.0 \pm 0.2 \cdot 10^9$ (650)		
PQH^{2+}•	Aqueous pH 7.4	7300 (400)	$7.4 \pm 0.6 \cdot 10^8$ (410)		
		1700 (550)			
	Aqueous pH 5.6	7300 (400)			
		1800 (550)	$7.1 \pm 0.5 \cdot 10^8$ (560)		
	Aqueous pH 2.3	6500 (410)			
		2000 (570)	$6.0 \pm 0.8 \cdot 10^8$ (560)		
PQ•- / PQH•	Aqueous pH 7.4	4700 (350)			
		2100 (490)	$2.0 \pm 0.2 \cdot 10^9$ (490)		
$^1O_2$*	Toluene			0.025 ($R^2 \geq 0.977$)	
PQ + ^{3}FEN* →	Acetonitrile				$3.0 \pm 0.5 \cdot 10^9$
PQ + ^{3}BP* →	Toluene				$3.9 \pm 0.5 \cdot 10^9$
PQ + Br_2•- →	Aqueous pH 10.8				$4.2 \pm 0.1 \cdot 10^9$
PQH_2^{2+} + Br_2•- →	Aqueous pH 2.4				$2.7 \pm 0.2 \cdot 10^9$
PQH^{+} + Br_2•- →	Aqueous pH 7.4				$4.7 \cdot 10^9$ ($R^2 = 0.998$)
PQH^{+} + OH• →	Aqueous pH 7.4				$6.6 \cdot 10^9$ ($R^2 = 0.957$)
PQH^{+} + TRP• →	Aqueous pH 7.4				$2.5 \pm 0.3 \cdot 10^7$
PQH^{+} + O_2•$^-$ ↛	Aqueous pH 7.4				No reaction
PQH^{2+}• + O_2 →	Aqueous pH 7.4				$1.0 \cdot 10^6$ ($R^2 = 0.962$)

Abbreviations: PQ = primaquine; FEN = fenbufen; BP = biphenyl; TRP = tryptophan; Є = molar absorption coefficient; λ_{max} = absorption maximum; k_2 = second order rate constant of transient decay, measured at wavelength λ; $^{PQ}\Phi_{\Delta}$ = quantum yield of $^1O_2$-formation sensitized by primaquine; k_q = bimolecular quenching rate constant.