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Photochemical stability of antimalarials. I. Hydroxychloroquine

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Summary

Hydroxychloroquine has been reported to cause toxic reactions which may be ascribed to the photochemical degradation of the substance. The possibility of photochemical dechlorination has also been indicated. The effect of light at a wavelength of 240–600 nm on aqueous solutions of hydroxychloroquine was investigated. The four decomposition products obtained were isolated by means of preparative TLC. The purity of the samples was examined by HPLC. The samples were identified by mass-spectrometry (EI, CI and high-resolution MS) and nuclear magnetic resonance spectroscopy (^{13}C- and ^{1}H-NMR). Under the given experimental conditions a photodechlorination of hydroxychloroquine was not observed.

Introduction

Hydroxychloroquine is one of a large series of 4-amino quinolines with antimalarial activity. It has been used in malaria therapy since 1955 as an alternative to or in combination with chloroquine, which only differs slightly in structure from hydroxychloroquine (Mills et al., 1981). Although hydroxychloroquine was developed primarily as an antimalarial agent, it possesses several other pharmacological properties as well. Its anti-inflammatory properties are well known and it has been useful in the treatment of rheumatoid arthritis and in systemic lupus erythematosus. Its applicability in the treatment of photo-allergic reactions is also established (Moore and Hemmens, 1982). Treatment of these conditions, however, requires the administration of much larger doses of the drug than are employed in prophylaxis and treatment of malaria (Goodman and Gilman, 1975). Hydroxychloroquine shows substantial binding to plasma proteins, and it binds to metabolically active tissues including the liver, spleen, lung and adrenal glands, where it accumulates with long-term administration, reaching concentrations of 6000–80,000 times the plasma level (Tanenbaum et al., 1980). It is also deposited in the epidermis in considerable amounts, at levels 100–200 times the plasma concentration. Further, hydroxychloroquine accumulates in melanin-rich areas of the body and is retained in the iris and choroid of the eye (Tanenbaum et al., 1980).

Several toxic side-effects are associated with the use of hydroxychloroquine. The drug have been reported to cause changes in skin-pigmentation and bleaching of the hair (Moore and Hemmens, 1982; Tanenbaum et al., 1980). With long-term usage, hydroxychloroquine is likely to affect the

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cornea and retina of the eye (Carr et al., 1968; Ehrenfeld et al., 1986; Finbloom et al., 1985; Mills et al., 1981). Hydroxychloroquine-induced retinopathy is dose-related, and rarely occurs for cumulative doses less than 200 g. Once it develops, it is usually stable and irreversible.

The cutaneous and ocular effects of hydroxychloroquine are possibly phototoxic reactions (Moore and Hemmens, 1982). Of the many drug substances reported to cause phototoxic reactions, a significant number contain chlorine. Photodechlorination is suggested to be the cause of the photosensitization observed with the administration of these drugs. It has been suggested that the free radical formed by the splitting of the C–Cl bond is able to combine readily with proteins, for instance, in the skin (Moore and Tamat, 1980). Chloroquine is shown to have a photosensitizing action as inducer of free radicals in DNA (Piette et al., 1978). The origin of free radicals from chloroquine is shown to be due to a photodechlorination process when the experiments are performed in deaerated systems (Moore and Hemmens, 1982). In vivo, however, the conditions will be different since oxygen is not excluded from tissues. It was therefore of interest to study the photochemical degradation of hydroxychloroquine in oxygen-containing media to investigate whether the possible phototoxic reactions of the drug can be purely ascribed to the presence of chlorine in the molecule.

Materials and Methods

Materials

Pure hydroxychloroquine sulphate was provided by Norsk Medisinaldepot, Norway. All the chemicals used were of p.a. grade.

Preparation of the samples

Irradiation in water. 4 g of hydroxychloroquine sulphate were dissolved in 490 ml of distilled water. After exposure to light, concentrated ammonia was added until the precipitation was completed. The sample was extracted with 4 × 100 ml chloroform, and evaporated to dryness under vacuum. The residue was dissolved in chloroform or methanol for further analysis.

Irradiation in isopropanol. 4 g of hydroxychloroquine sulphate were dissolved in 75 ml of distilled water. Concentrated ammonia was added until the precipitation was completed. The sample was extracted with 1 × 100 and 3 × 50 ml of chloroform. The combined extracts were washed with 50 ml water and dried by addition of sodium sulphate before evaporation to dryness under vacuum. The residue was dissolved in 490 ml isopropanol. After exposure to light, the sample was evaporated under vacuum. The residue was dissolved in methanol or chloroform for further analysis.

The photochemical degradation of hydroxychloroquine was followed by HPLC.

After irradiation, the photochemical degradation products were isolated by means of preparative TLC. The samples were extracted from the silica gel with chloroform or methanol. The purity of the isolated fractions were controlled with HPLC before further identification was carried out by mass spectrometry (EI, CI and high resolution mass spectrometry) and NMR (^{13}C- and ^{1}H-).

Preparative TLC

Stationary phase was silica gel (Merck), mobile phase was methanol/ammonia (100 : 3).

HPLC analysis

For HPLC analysis a Spectra-Physics SP 8700 liquid chromatograph was used. The injector was a Shimadzu Sil-6A auto-injector. The analysis was carried out at ambient temperature. The detector was a LDC Spectro-Monitor III, detection wavelength 340 nm. The stationary phase was Bondapak C-18 (Waters), particle size 10 μm, pre-packed in a 300 mm × 3.9 mm i.d. column. The mobile phase was acetonitrile/methanol/ammonia (65 : 32.5 : 2.5).

Irradiation

The samples were exposed to an immersion lamp with emission wavelengths of 240–600 nm, 120 W (Hereaus immersion lamp system).

Mass spectrometry

Electron-impact mass spectra and chemical-ionization mass spectra (ionization gas: isobutane) were obtained with a VG Micromass 7070 F mass spectrometer via direct inlet. The probe temperature was 220 °C and the ion potential was 70 eV. High-resolution mass spectra were recorded on a AEI MS 902.

NMR

The NMR-spectra were recorded on a Bruker CXP 200.

Results and Discussion

An HPLC chromatogram of hydroxychloroquine irradiated in isopropanol for 5 h is given in Fig. 1.

The photochemical decomposition reactions went faster in solutions of isopropanol than when hydroxychloroquine was dissolved in water. Isopropanol was therefore chosen as the reaction medium for the isolation of the degradation products, since the degradation patterns of hydroxychloroquine in the two solvents were shown to be qualitatively identical.

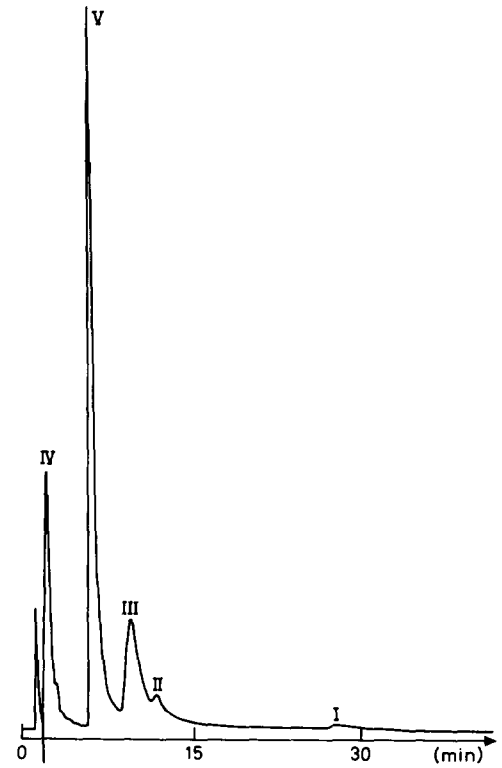


Fig. 1. HPLC chromatogram of hydroxychloroquine in isopropanol after exposure to light (240–600 nm) for 5 h. Peak numbers I–IV refers to compounds I–IV (Fig. 2). Peak number V is hydroxychloroquine.

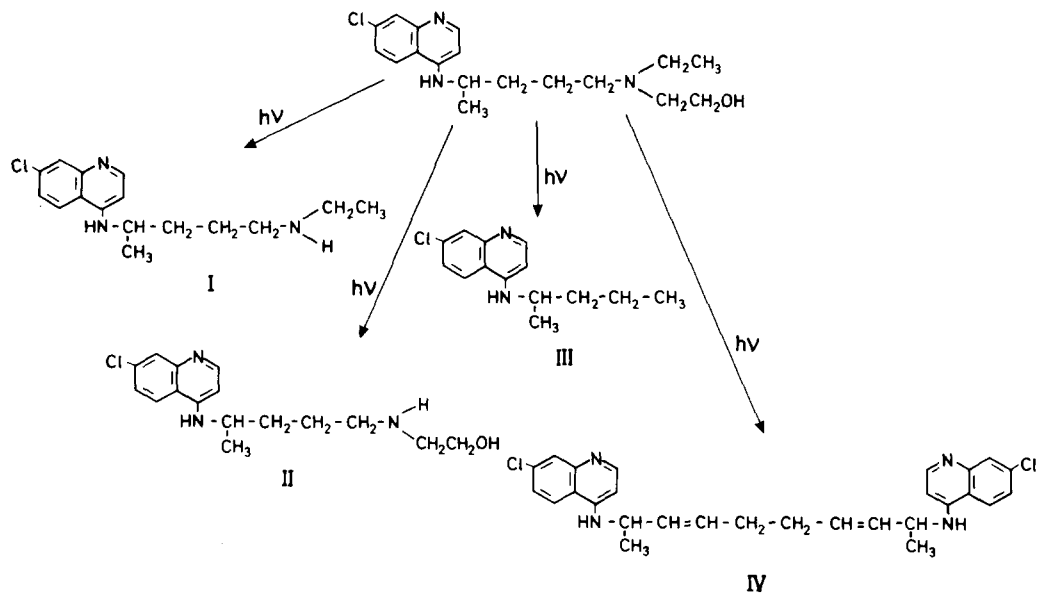
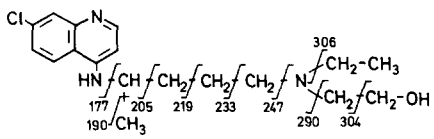


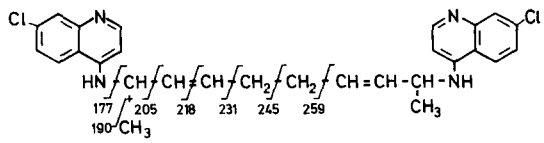
Fig. 2. Postulated degradation pattern of hydroxychloroquine in water or isopropanol after exposure to light (240–600 nm).

TABLE 1

EI mass fragments (m/e) of hydroxychloroquine and its isolated photodegradation products



Hydroxychloroquine



Compound 4

Hydroxychloroquine			Compound I		Compound II		Compound III		Compound IV	
m/e	%	fragment	m/e	%	m/e	%	m/e	%	m/e	%
336	2.9	$M^{+}+1$								
306	16.1	$M-C_2H_5^{\cdot+}$			307	2.3				
304	39.6	$M-CH_2OH^{\cdot+}$	291	0.7						
290	1.0	$M-C_2H_4OH^{\cdot+}$			290	0.9				
247	60.5	$M-NC_4H_9OH^{\cdot+}$	247	3.2	247	3.2	247	3.6	247	4.4
									245	5.7
233	2.8	$M-CH_2NC_4H_9OH^{\cdot+}$	233	0.9	233	3.6				
219	3.6	$M-C_2H_4NC_4H_9OH^{\cdot+}$	219	4.1	219	11.8	219	1.4	219	14.2
205	12.5	$M-C_3H_6NC_4H_9OH^{\cdot+}$	205	6.9	205	22.9	205	100.0	205	44.2
191	14.5	$M-C_4H_8NC_4H_9OH^{\cdot+}$	191	0.3	191	2.3	190	4.6	190	5.2
179	13.0	$M-C_5H_{10}NC_4H_9OH^{\cdot+}$	179	16.4	179	47.4	179	20.3	179	13.5
Other peaks			*Other peaks*		*Other peaks*		*Other peaks*		*Other peaks*	
245	56.4		163	2.0	276	24.3	229	6.0	231	7.3
126	18.0		135	1.3	169	7.1	170	22.2	170	8.6
114	6.3		112	2.8	162	5.5	162	7.5	162	5.5
112	6.8		98	2.9	98	41.5	155	7.3	155	4.0
102	100.0		44	100.0	44	100.0	135	6.8	135	3.4
99	8.3								28	100.0

The irradiation of hydroxychloroquine in water or in isopropanol resulted in 4 degradation products (Fig. 2, Tables 1–3). Two of the compounds were identified as the desethyl- and desethanol hydroxychloroquine, respectively (I, II). Compared to metabolic studies of chloroquine (Florey, 1984), compound I and II are also likely to be formed by metabolism of hydroxychloroquine in vivo. The third product was identified as hydroxychloroquine without the $N-C_4H_{10}O$ part of the substituent in the C-10 position (III). The last product was found to be a dimer of this compound (IV). In water, the dimer was formed to a lesser extent than in isopropanol. The chlorine-atom was intact in the C-15 position of the molecule in all the degradation products formed during irradiation.

The photochemical degradation of hydroxychloroquine in vitro indicates that the postulated phototoxicity of the compound in vivo cannot be

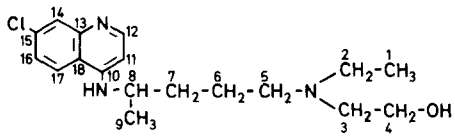
TABLE 2

High-resolution values of mass fragments from the photodegradation products of hydroxychloroquine

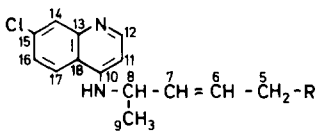
Compound I	Compound 2	Compound 3	Compound 4
219: $C_{12}H_{12}N_2Cl$	179: $C_9H_8N_2Cl$	304: $C_{17}H_{23}N_3Cl$	245: $C_{14}H_{14}N_2Cl$
102: $C_5H_{12}NO$		205: $C_{11}H_{10}N_2Cl$	231: $C_{13}H_{14}N_2Cl$

TABLE 3

^{13}C-NMR shifts of hydroxychloroquine and its photodecomposition products (the amount of compound 1 was insufficient to make this spectrum)



Hydroxychloroquine



Compound 4

Hydroxy-chloroquine	Compound 2	Compound 3	Compound 4
C_1 : 11.685			
C_2 : 47.469			
C_3 : 61.934	60.290		
C_4 : 59.251	59.215		
C_5 : 53.327	53.259	19.528	19.651
C_6 : 23.597	23.536	23.606	123.678
C_7 : 33.259	33.288	33.284	123.900
C_8 : 47.598	47.584	47.575	47.469
C_9 : 19.761	19.745	19.783	19.793
C_{10}: 149.446	149.689	149.472	149.495
C_{11}: 98.757	98.725	98.818	98.789
C_{12}: 151.793	151.793	151.867	151.761
C_{13}: 149.236	149.450	149.262	149.142
C_{14}: 127.371	127.283	127.381	127.277
C_{15}: 133.262	133.237	133.291	133.294
C_{16}: 123.682	123.665	123.739	123.753
C_{17}: 124.280	124.251	124.374	124.381
C_{18}: 117.467	117.398	117.561	117.482

ascribed to a simple photodechlorination of the molecule but possibly involves other reactions.

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