Hydroxyl and hydroperoxy radical scavenging by copper(II) chelates related to non-steroidal anti-inflammatory drugs

M.R. Billany, Joanne McMullan, Claire Ranson, J.K. Sugden *

Department of Pharmaceutical Sciences, School of Applied Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK

Abstract

Solutions of dequalinium chloride with and without added hydrogen peroxide were irradiated in buffer solutions with simulated sunlight at $23 \pm 1°$ C. The photodegradation of dequalinium chloride solution and hydrogen peroxide in buffer at pH 7.0 and 10.7 with the copper(II) chelates of ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylates and 3,5-diisopropylsalicylic acid showed that only the copper(II) chelate of the latter scavenged hydroxyl radicals at pH 7.0. All the compounds tested showed hydroxyl radical scavenging effects at pH 10.7 and were scavengers for the hydroperoxy radical at pH 7.0. The structure-activity relationships in the compounds tested are discussed.

1. Introduction

Parkash et al. (1993) showed that aspirin, ibuprofen and phenylbutazone have the ability to scavenge hydroxyl radicals in an aqueous model system consisting of dequalinium chloride and hydrogen peroxide on irradiation with simulated sunlight, by the method of Evans et al. (1975). The test drugs in that work were solubilised by the addition of 2% w/v ethanol and the reaction was found to follow second-order kinetics; ibuprofen was shown to be the most active of the drugs tested.

Sorenson (1984) observed that the natural biological antioxidant, superoxide dismutase, was a copper(II) derivative with the metal ion forming part of a chelate. He suggested that copper chelates may be effective in the treatment of animal models of arthritic disease and that copper(II) ions with a suitable ligand were prerequisites for the in vivo formation of a compound to exert antirheumatic activity (Sorenson, 1976). Oberley and Beuttner (1979) investigated the copper(II) ligands of substituted salicylic acids for superoxide dismutase activity and found that 3,5-diisopropylsalicylic acid as its copper(II) chelate was the most effective of the compounds tested.

The object of the present work is to investigate the hydroxyl radical scavenging properties of other

* Corresponding author.

copper(II) chelates in a model system in which their effects on the rate of degradation, by photo-generated hydroxyl radicals, on dequalinium chloride are measured.

2. Materials and methods

2.1. Materials

The following materials were obtained from the indicated sources: aspirin (BDH, batch no. 9983970), buffer solution tablets (BDH, batch no. 20543021, 330400130), copper(II) sulphate pentahydrate (BDH, batch no. 227334), ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylates (De Montfort University, Leicester), dequalinium chloride (Sigma Chemical Co., batch no. 25889R), 3,5-diisopropylsalicylic acid (Sigma Chemical Co., batch no. 13569-0), ethanol (Fisons Bulk Supplies, batch no. 4765370H) hydrogen peroxide (BDH, batch no. 80H0303), and sodium hydroxide (Fisons, batch no. 23220331).

2.2. Apparatus

A Perkin Elmer 550 spectrophotometer with matched silica cells of 1 cm path length was used.

2.3. Methods

Solutions of dequalinium chloride (0.0192 g) were made up in buffer solution (500 ml). Serial dilutions were prepared from this stock, the absorbance measured at 326 nm and a calibration graph constructed. Linear regression analysis showed that the regression coefficient was 0.9988 ($p = 0.001$). The stock solution was diluted with deionised water to give a solution containing 0.00384 g/l of dequalinium chloride. The copper(II) chelates of ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylates were prepared from copper(II) sulphate pentahydrate and the appropriate ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylate in solution according to the method of Wilson et al. (1988) using 2 mol of ligand per mol of copper(II) sulphate pentahydrate. Equimolar amounts of the chelate solutions and hydrogen peroxide solution were added to aliquots of the dequalinium chloride solution to give a final volume of 100 ml. Control solutions were prepared without the chelates, with and without the hydrogen peroxide. All the solutions tested contained ethanol (2% v/v) which was used to solubilise some of the compounds. Each reaction mixture was irradiated by the method of Evans et al. (1975) at $23 \pm 1°$C and

Table 1
Photodegradation of dequalinium chloride (0.384 mg/ml) with hydrogen peroxide (1 ml, 3%)

Time (h)	Absorbance	% C remaining	Log % DC remaining	1/% DC remaining
0	1.1650	100.00	2.0000	0.01000
1	0.9045	77.64	1.8900	0.01287
2	0.7525	64.59	1.8102	0.01548
3	0.6695	57.47	1.7594	0.01740
4	0.6065	52.06	1.7165	0.01920
5	0.5715	49.05	1.6906	0.02038
		$r = -0.942$	$r = -0.970$	$r = 0.989$
			steps × 10	steps × 1000
			1 = 1.100	1 = 2.87
			2 = 0.598	2 = 2.61
			3 = 0.508	3 = 1.92
			4 = 0.429	4 = 1.80
			5 = 0.259	5 = 1.18
			SD = 0.31	SD = 0.67
			first-order slope = 0.0605	
			rate constant = 13.93×10^{-2}	

Irradiation of dequalinium chloride solution (0.0264 mg/ml) without hydrogen peroxide gave no reaction under the conditions of test.

Table 2
Photodegradation of dequalinium chloride (0.384 mg /ml) in buffers

Substrate	pH of buffer	Order of reaction	Rate constant
DC in buffer	7.0	first	0.520×10^{-2}
DC in buffer	8.4	no reaction	–
DC in buffer	9.2	no reaction	–
DC in buffer	10.6	first	2.20×10^{-2}
DC + H_2O_2 in buffer	7.0	first	20.4×10^{-2}
DC + H_2O_2 in buffer	8.4	first	27.59×10^{-2}
DC + H_2O_2 in buffer	9.2	first	4.49×10^{-2}
DC + H_2O_2 in buffer	10.6	first	7.85×10^{-2}

absorbance readings taken at 326 nm at time zero and regular intervals thereafter up to 5 h.

2.4. Treatment of results

The reaction order was determined by carrying out a linear regression analysis of plots of the percentage of dequalinium chloride remaining against time, log percentage of dequalinium chloride remaining against time and the reciprocal of the percentage of dequalinium chloride remaining against time. The plot yielding the best straight line as determined by the method of Patel and Sugden (1992) was deemed to represent the order

of reaction and the rate constant calculated therefrom.

3. Discussion

Examination of Table 1 shows that irradiation of a dequalinium chloride solution (0.0264 mg/ml) in deionised water without hydrogen peroxide gave no measurable degradation over a 5 h period. However, the photodegradation of dequalinium chloride and hydrogen peroxide in deionised water gave a rate constant of 13.95 ×

Table 3
Photodegradation of dequalinium chloride (0.384 mg/ml) with additives at pH 7.0

Substrate	Order of reaction	Rate constant
Control DC + H_2O_2	first	20.4×10^{-2}
DC + H_2O_2 + Cu DIPS	first	2.67×10^{-2}
DC + H_2O_2 + Cu Et chelate [a]	first	26.9×10^{-2}
DC + H_2O_2 + Cu Pr^i chelate [a]	first	20.47×10^{-2}
DC + H_2O_2 + Cu Bu^t chelate [a]	first	22.04×10^{-2}
DC + Cu(II)Et chelate [a] + ABAP	no reaction	
DC + Cu(II)Pr^i chelate [a] + ABAP	no reaction	
DC + Cu(II)Bu^t chelate [a] + ABAP	no reaction	
DC + DIPS [b] + ABAP	no reaction	
DC + Cu(II)DIPS [c] + ABAP	no reaction	
DC + aspirin + ABAP	no reaction	
DC + ibuprofen + ABAP	no reaction	
DC + salicylic acid + ABAP	no reaction	
DC + ABAP	first	0.527×10^{-2}
Control DC + H_2O_2 + Cu^{2+}	first	67.4×10^{-2}

[a] Copper(II) chelates of ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylates.
[b] DIPS = 3,5-diisopropylsalicylic acid.
[c] Copper chelate of 3,5-diisopropylsalicylic acid.

10^{-2} and followed first-order kinetics as did the work of Patel and Sugden (1992).

Examination of Table 2 shows that the pH of the solution has a profound effect on the rate of reaction both with and without the addition of hydrogen peroxide.

Table 2 shows that the rate constant of the photochemical degradation of dequalinium chloride in the presence of hydrogen peroxide is markedly influenced by the pH of the solution. However, there was no change in the order of the reaction.

Clearly, it is important to control the pH at which the experiment is conducted in order to obtain consistent results.

Table 3 shows the effect on the rate constants of the photochemical degradation of dequalinium chloride in buffer solution with the addition of hydrogen peroxide at pH 7.0. Of the compounds tested only the copper(II) chelate of 3,5-diisopropylsalicylic acid [copper(II)DIPS] had any hydroxyl radical scavenging action. This was of interest in view of the work of Whitehouse (1962) who postulated that in order for a molecule to have antirheumatic activity it must have a lipophilic character and the ability to form complexes with metal ions. The planar structures of the ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylates has been demonstrated by studies of molecular models (Beckett et al., 1965). These compounds were shown to have lipophilic character and are able to form chelates with transition metals, but their antirheumatic activity was poor when tested on animals (Beckett et al., 1965). However, in this case there were no significant changes in the rate constants for the photochemical reactions of the pyrroline copper(II) chelates tested. This indicated that the compounds tested had no hydroxyl radical scavenging activity at pH 7.0. The effect of the copper ions alone in this system did have a substantial hydroxyl radical promoting effect as might be expected from consideration of the Fenton reaction. Copper(II) ions have been reported to have mild oxidative properties (Walling and Weil (1974) and such activity was manifest in this experiment. There was clear evidence that hydroperoxy radicals (HO_2), generated by the substitution of hydrogen peroxide for 2,2'-azobis(2-amidinopropane) dihydrochloride (ABAP), were completely scavenged by the copper(II) chelates of ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylates, since the dequalinium chloride did not undergo photochemical decomposition whereas the control reaction showed a rate constant of 0.527×10^{-2}, and followed first-order kinetics.

The results from the experiments using ABAP (hydroperoxy radicals) were of particular interest in view of the relationship between the hydroperoxy and hydroxyl radicals:

$$H_2O_2 \xrightarrow{h\nu} 2\,OH^{\cdot} + H^+$$
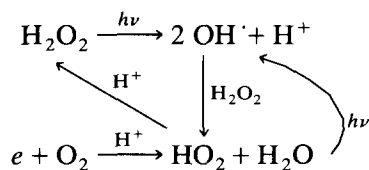
(Ho et al., 1994)

The data in Table 3 suggest that this test for hydroperoxy radical scavenging is inappropriate for distinguishing potential antirheumatic drugs from those compounds with modest activity.

Table 4
Photodegradation of dequalinium chloride (0.384 mg/ml) with additives at pH 10.7

Substrate	Order of reaction	Rate constant
Control DC + H_2O_2	first	18.3×10^{-2}
DC + H_2O_2 + Cu(II)DIPS chelate [b]	first	0.203×10^{-2}
DC + H_2O_2 + Cu(II)Et chelate [a]	first	3.22×10^{-2}
DC + H_2O_2 + Cu(II)Pr^i chelate [a]	first	0.94×10^{-2}
DC + H_2O_2 + Cu(II)Bu^t chelate [a]	first	2.92×10^{-2}
DC + H_2O_2 + aspirin	first	10.36×10^{-2}

[a] Copper(II) chelates of ethyl N-substituted 4-hydroxy-5-oxo-3-pyrroline-3-carboxylates.
[b] DIPS = 3,5-diisopropylsalicylic acid.

Consideration of the information in Table 4 would suggest that the hydroxyl radical scavenging activity of the copper(II) chelates of the pyrroline derivatives was dependent on the pH of the test medium, but this pH dependence was absent in the case of DIPS.

Examination of Table 4. shows that at pH 10.7 all of the four copper(II) chelates tested were effective hydroxyl radical scavengers. The isopropyl derivative showed optimal activity amongst the pyrroline derivatives but it was less active than the copper(II) chelate of DIPS and all of those tested were superior to aspirin under these conditions. It is of interest that DIPS has an aromatic structure, a phenolic hydroxy and a carboxylic acid group as well as two isopropyl groups and this compound, as its copper(II) chelate, exhibits significant hydroxyl radical scavenging activity, irrespective of the pH of the medium. Sorenson (1984) reported that this compound mimics the physiological properties of the enzyme superoxide dismutase (SOD). However, at pH 10.7, these results would have no physiological implications and in this respect the results obtained at pH 7.0 are the more realistic. It could be suggested that at physiological pH the structural requirements for antirheumatic activity would appear to be an aromatic structured ligand rather than a substantially planar molecule containing a π-electron system with chelating ability with respect to copper(II) ions. In addition, there is some indication from the results reported on ibuprofen, DIPS and aspirin (Parkash et al., 1993) to be a need for a carboxylic acid group in the molecule. However, the carboxylic acid group is absent in phenylbutazone which has hydroxyl radical scavenging activity comparable to that of aspirin in the test procedure used by these authors. Attempts to highlight structure-activity relationships in non-steroidal antirheumatic drugs may be inappropriate if the key feature is the ability to scavenge hydroxyl radicals since this feature is present, to varying degrees, in diverse chemical structures. This work emphasises the need to carry out tests for hydroxyl radical scavenging at physiological pH in order to discriminate between potentially useful compounds and those of minimal activity.

References

Beckett, A.H., Lee, C.M. and Sugden, J.K., Some pyrroline derivatives as potential anti-inflammatory agents. *J. Pharm. Pharmacol.*, 17 (1965) 498–503.

Evans, P.G.E., Sugden, J.K. and Van Abbe, N.J., Aspects of the photochemical behaviour of 1-hydroxypyridine-2-thione. *Pharm. Acta Helv.*, 50 (1975) 94–99.

Ho, A.H.L., Popat, S.C. and Sugden, J.K., Some effect of metal ions on the photogeneration of hydroxyl radicals. *Int. J. Pharm.*, 104 (1994) 247–251.

Oberley, L.W. and Beuttner, G.R., Role of superoxide dismutase in cancer. *Cancer Res.*, 39 (1979) 1141–1143.

Parkash, A.K., Sugden, J.K., Yucan Z. and Mingxin, W., A rapid method for screening non steroidal anti-inflammatory drugs for hydroxyl scavenging. *Int. J. Pharm.*, 92 (1993) 151–155.

Patel, R. and Sugden, J.K., Photodegradaton of aqueous solutions of dequalinium chloride. *Pharmazie*, 47 (1992) 113–115.

Sorenson, J.R.J., Copper chelates as possible active forms of antiarthritic agents. *J. Med. Chem.*, 19 (1976) 135–148.

Sorenson, J.R.J., Copper complexes in biochemistry and pharmacology, *Chem. Br.*, 20 (1984) 1110–1113.

Walling, C. and Weil, T., Ferric ion catalysed decomposition of hydrogen peroxide in perchloric acid solution. *Int. J. Chem. Kinet.*, 6 (1974) 507–516.

Whitehouse, M.W., Structure activity relationships among drugs acting on connective tissue (antirheumatic drugs). *Nature*, 194 (1962) 984–985.

Wilson, C.J., Aulton, M.E., Patterson, L.H., Sugden, J.K. and Taylor, M.J., Macrophages as a potential drug delivery system. *J. Pharm Pharmacol.*, 40 (1988) 28P.