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A rapid method for screening non steroidal anti-inflammatory agents for hydroxyl radical scavenging

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Summary

Dequalinium chloride undergoes photodegradation in simulated sunlight at 22 ± 1°C. The reaction follows first order kinetics, the addition of a molar equivalent of hydrogen peroxide markedly increases the rate constant of this reaction. The incorporation of non-steroidal anti-inflammatory agents as well as hydrogen peroxide results in a diminution in the rate constant. In such cases where the test drug is insoluble in water the addition of 2% ethanol to effect solubilisation changes the order of reaction to second order but still provides a means of comparing the hydroxyl radical scavenging properties of the drugs. This technique is rapid and cheap and could form a feature of the standard screening technique for novel medicinal agents.

Introduction

Recent work has shown that many anti-inflammatory agents have the property of acting as hydroxyl radical scavengers (Pronai et al., 1990; Udassin et al., 1991). The anti-inflammatory drug, ibuprofen has been shown to prevent the formation of iron mediated generation of oxidants and iron mediated lipid peroxidation (Kennedy et al., 1990). 5-Aminosalicylic acid has also been shown to inhibit the formation of of hydroxyl radicals from a Fenton process and this effect has been discussed in the context of clinical treatment of ulcerative colitis with this compound (Grisham, 1990). The anti-ulcer agent, cimetidine, although chemically different from the drugs discussed above, has been shown to have hydroxyl radical scavenging properties (Uchida and Kawakishi, 1990).

Earlier workers have demonstrated a relationship between superoxide scavenging properties and anti-inflammatory activity in *E*-4-phenyl-3-buten-2-ones and they have also shown that there is no clear correlation between anti-inflammatory activity and hydroxyl radical scavenging activity (Saldanha et al., 1990). Benzoic acid esters have also been shown to have hydroxyl radical scaveng-

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ing properties when evaluated by an ESR method. The hydroxyl radical scavenging activity decreases with increase in the length of the ester chain length. It was considered worthy of note that the antipsoriatic effect of these esters increased as with increase in chain length (Hesselhoff et al., 1990).

The objective of the present work is to describe a novel technique for screening non-steroidal anti-inflammatory agents and related compounds for hydroxyl radical scavenging properties. This is effected by measuring the loss of dequalinium chloride and calculating the rate constant in a model system in which dequalinium chloride in deionised water with stoichiometric amounts of hydrogen peroxide are irradiated with simulated sunlight with and without the addition of the test drugs in stoichiometric amounts.

Materials and Methods

Materials

Dequalinium chloride (Sigma), ibuprofen (Sigma), phenylbutazone (Sigma), acetylsalicylic acid (BDH), salicylic acid (Analytical Supplies, Derby), 3,5-diisopropylsalicylic acid (Aldrich Chemical Co. Ltd) and hydrogen peroxide (100 volume) (Boots plc) were obtained from the indicated sources.

Methods

The UV/Vis spectrum of dequalinium chloride in deionised water was run against a blank in the same solvent and the absorbance maximum occurred at 326 nm. A calibration graph was constructed using a concentration range of 10–80 μg/ml, which obeyed the Beer Lambert law (correlation coefficient $r = 0.9976$, $p = 0.001$).

Samples of dequalinium chloride (37.8 μg/ml) in deionised water were irradiated at 22 ± 1°C with simulated sunlight according to the method of Evans et al. (1975) for up to 178 h. The absorbance at 326 nm was recorded at time intervals and the percentage of dequalinium chloride remaining was calculated. The experiment was repeated with 0.1 ml (100 volume)/10 ml of solution of hydrogen peroxide and then with acetylsalicylic acid and salicylic acid.

In the cases of ibuprofen and phenylbutazone the drugs were insufficiently soluble in water, so 2% ethanol was used and the experiment run again. The same modification of procedure was used for 3,5-diisopropylsalicylic acid.

Readings were taken on a Beckman DU70 spectrophotometer. In each case the order and rate constant of the reaction were calculated.

Treatment of results

The order of reaction and the rate constant were calculated by conducting a linear regression analysis on a Casio FX 7300 microcomputer on plots of the amount of dequalinium chloride remaining vs time, the $\log_{10}$ of the percentage dequalinium chloride remaining vs time and the reciprocal of the percentage dequalinium chloride remaining vs time. The analysis giving the highest regression coefficient was deemed to represent the order of reaction (Florence and Attwood, 1981).

Results and Discussion

Examination of Table 1 shows that the photodegradation of dequalinium chloride in deionised water follows first order kinetics with a rate constant of 5.3×10^{-3}. The addition of a stoichiometric amount of hydrogen peroxide resulted in an increase in the rate constant to 14.3×10^{-3}. This indicated that the reaction is probably hydroxyl radical mediated (Dorfman and Adams, 1973; Patel and Sugden, 1992). Table 2 shows that the irradiation of salicylic acid, dequalinium chloride and hydrogen peroxide is also a first order reaction and that the rate constant has been reduced to 11.2×10^{-3}. This indicates that salicylic acid has some hydroxyl radical scavenging property but that it is either insufficient to negate the effect of the added hydrogen peroxide or that the choice of stoichiometric amounts of the drugs was inadequate to achieve this objective. Table 3 shows the the use of acetylsalicylic acid under the same conditions also gives a first order reaction but the rate constant is $13.1 \times$

TABLE 1

Photochemical degradation of dequalinium chloride (DC) in deionised water (37.8 μg/ml)

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	1.765	100.00	2.0000	0.01000
2	1.759	99.7	1.9987	0.01003
44	1.434	81.2	1.9096	0.01231
50	1.311	74.2	1.8704	0.01348
68	1.219	69.0	1.8388	0.01449
92	1.147	64.9	1.8122	0.01541
98	1.039	58.2	1.7698	0.01717
154	0.781	44.2	1.6454	0.02262
178	0.682	38.6	1.5870	0.02591
r		−0.981	−0.995	0.978

Reaction follows first order kinetics
$k = 5.3 \times 10^{-3}$

Addition of 0.1 ml hydrogen peroxide (100 volume)

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	1.851	100.00	2.0000	0.01000
1	1.821	98.34	1.9927	0.01017
2	1.789	96.65	1.9852	0.01034
4	1.739	93.95	1.9729	0.01064
24	1.147	61.96	1.7921	0.01614
48	0.774	41.84	1.6216	0.02390
72	0.578	31.25	1.4948	0.03200
96	0.464	25.09	1.3995	0.03985
168	0.310	16.75	1.2240	0.06154
r		−0.902	−0.967	0.955

Reaction follows first order kinetics
$k = 14.3 \times 10^{-3}$

TABLE 2

Photochemical degradation of dequalinium chloride (37.8 μg/ml) with salicylic acid (9.9 μg/ml) and hydrogen peroxide (0.1 ml, 100 volume)

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	1.963	100.00	2.0000	0.01000
1	1.918	97.71	1.9899	0.01020
2	1.892	96.38	1.9839	0.01037
4	1.878	95.69	1.9809	0.01045
24	1.480	75.39	1.8773	0.01330
48	1.158	59.00	1.7709	0.01695
72	0.880	44.84	1.6517	0.02230
96	0.698	35.55	1.5509	0.02810
r		−0.970	−0.999	0.992

Reaction follows first order kinetics
$k = 11.2 \times 10^{-3}$

TABLE 3

Photochemical degradation of dequalinium chloride (37.8 μg/ml) with acetylsalicylic acid (12.9 μg/ml) and hydrogen peroxide (0.1 ml, 100 volume)

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	3.137	100.00	2.0000	0.01000
1	3.091	98.53	1.9936	0.01015
2	2.999	95.60	1.9805	0.01046
4	2.814	89.70	1.9528	0.01115
24	1.545	49.25	1.6824	0.02030
28	1.410	44.96	1.6528	0.02224
48	1.067	33.73	1.5279	0.02964
52	1.011	32.22	1.5081	0.03104
72	0.841	26.81	1.4283	0.03729
120	0.509	15.08	1.1785	0.06631
144	0.439	13.98	1.1456	0.07153
168	0.348	11.10	1.0454	0.09009
r		−0.863	−0.967	0.961

Reaction follows first order kinetics
$k = 13.1 \times 10^{-3}$

10^{-3}. This might suggest that the acetyl derivative is less active than the parent acid but also has some hydroxyl radical scavenging activity.

In the second series of experiments, the drugs were solubilised by the addition of ethanol giving overall 2% in the final solution. Examination of Tables 4–6 shows that where ethanol is used, the

TABLE 4

Photochemical degradation of dequalinium chloride (37.8 μg/ml) with ibuprofen (31.7 μg/ml) and hydrogen peroxide (0.1 ml, 100 volumes) in 2% ethanol

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	1.50	100.00	2.0000	0.0100
1	0.90	59.60	1.7752	0.01682
2	0.76	50.33	1.7018	0.01980
3	0.69	45.69	1.6598	0.02190
4	0.58	38.41	1.5844	0.02600
5	0.53	35.09	1.5452	0.02850
6	0.47	33.11	1.5199	0.03020
7	0.468	31.12	1.4931	0.03210
24	0.18	11.92	1.0763	0.08390
r		−0.714	−0.924	0.998

Reaction follows second order kinetics
$k = 2.9 \times 10^{-3}$

TABLE 5

Photochemical degradation of dequalinium chloride (37.8 μg/ml) with phenylbutazone (11.6 μg/ml) and hydrogen peroxide (0.1 ml, 100 volume) in 2% ethanol

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	1.39	100.00	2.0000	0.0100
1	1.06	76.26	1.8323	0.01310
2	0.87	62.59	1.7965	0.01590
3	0.80	57.55	1.7600	0.01740
4	0.74	53.23	1.7261	0.01880
5.16	0.70	50.36	1.7021	0.01980
6	0.69	49.64	1.6858	0.02014
r		−0.896	−0.932	0.961

Reaction follows second order kinetics
$k = 3.6 \times 10^{-3}$

reaction follows second order kinetics. This is not surprising since ethanol will take part in the reaction as it is a potent hydroxyl radical scavenger (Tadolini and Cabrini, 1988). Under these experimental conditions, the rate constant for acetylsalicylic acid has a value of $3.38 \times 10^{\circ 3}$, that for ibuprofen of 2.9×10^{-3} and that for phenylbutazone a value of 3.6×10^{-3}. A control experiment in which the drugs were omitted and only ethanol at 2% was used gave a rate constant of 4.96×10^{-3}. These results indicate a relative order of hydroxyl radical scavenging for these drugs. Ibuprofen appears to be the most active of the three drugs tested and this result mirrors the pharmacological findings of Kennedy et al. (1990).

TABLE 6

Photochemical degradation of dequalinium chloride (37.8 μg/ml) with acetylsalicylic acid (12.8 μg/ml) and hydrogen peroxide (0.1 ml, 100 volume) in 2% ethanol

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	1.44	100.00	2.0000	0.0100
1	1.04	72.22	1.8586	0.01385
1.83	0.96	66.67	1.8239	0.01500
3.16	0.85	59.03	1.7710	0.01694
4.80	0.76	50.69	1.7050	0.01972
6.71	0.69	47.92	1.6804	0.02090
r		−0.896	−0.941	0.973

Reaction follows second order kinetics
$k = 3.38 \times 10^{-3}$

TABLE 7

Photochemical degradation of dequalinium chloride (37.8 μg/ml) and diisopropylsalicylic acid (15.9 μg/ml) with hydrogen peroxide (0.1 ml, 100 volume) in 2% ethanol

Time (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	1.832	100.00	2.0000	0.0100
1	1.756	95.85	1.9816	0.01043
2	1.703	92.96	1.9683	0.01075
4	1.622	88.54	1.9471	0.01129
24	1.020	55.67	1.7456	0.01796
28	0.954	52.07	1.7166	0.01920
48	0.691	41.11	1.6139	0.02432
72	0.545	29.75	1.4735	0.03361
142	0.320	17.46	1.2420	0.05727
r		−0.888	−0.970	0.996

Reaction follows second order kinetics
$k = 7.39 \times 10^{1}$

Table 7 shows the results from testing 3,5-diisopropylsalicylic (DIPS). The reaction follows second order kinetics, as would be expected with a substance which needs the addition of ethanol to effect solubility in the test solvent. There was a massive increase in the rate constant, suggestive of the compound facilitating the degradation of the model compound, dequalinium chloride. The diisopropyl analogue gave a rate constant of 7.39×10^{1}. It is worthy of note that Sorenson (1984) described DIPS as being, in the form of its Cu(II) chelate, a substitute for superoxide dismutase (SOD). This enzyme acts on the superoxide anion radical:

$$2O_2^{-} + 2H^{+} + (SOD) = O_2 + H_2O_2$$

The presence of SOD facilitates hydrogen peroxide formation in vivo and this is then decomposed by other enzymes. If DIPS has a similar action in the system used in the present work it may afford some explanation for the results obtained. However, further work would be needed to establish this point.

The main advantage of the technique described above is that it affords a rapid screening method for compounds which may be active in scavenging hydroxyl radicals. This property is becoming of increasing interest as there are reports that β-blockers and drugs which modify Ca^{2+} metabolism have the property of scavenging hydroxyl radicals (Aruoma et al., 1991). There is an increasing amount of evidence to demonstrate that the hydroxyl radical generated in vivo can, if not decomposed, produce serious harmful effects.

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