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CHARACTERIZATION OF THE POTENTIAL ANTIOXIDANT AND PRO-OXIDANT ACTIONS OF SOME NEUROLEPTIC DRUGS

INGE JEDING, PATRICIA J. EVANS, DOLA AKANMU, DAVID DEXTER, JEREMY D. SPENCER, OKEZIE I. ARUOMA, PETER JENNER and BARRY HALLIWELL*

Neurodegenerative Disease Research Centre, Pharmacology Group, King's College, Manresa Road, London SW3 6LX, U.K.

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Abstract—It has been suggested in the literature that neuroleptic drugs may be able to exert antioxidant and/or pro-oxidant actions *in vivo*. The feasibility of this was tested by measuring the ability of chlorpromazine, prochlorperazine, metoclopramide, methotrimeprazine and haloperidol to scavenge biologically relevant oxygen-derived species *in vitro*. None of the drugs reacted with superoxide radical at a significant rate. Chlorpromazine, prochlorperazine, metoclopramide and methotrimeprazine were very powerful scavengers of hydroxyl radicals, reacting at almost a diffusion-controlled rate. Chlorpromazine showed some ability to inhibit iron ion-dependent hydroxyl radical formation. Chlorpromazine, methotrimeprazine, promethazine and prochlorperazine were powerful inhibitors of iron ion-dependent liposomal lipid peroxidation, scavengers of organic peroxyl radicals and inhibitors of haem protein/hydrogen peroxide-dependent peroxidation of arachidonic acid. Chlorpromazine, prochlorperazine, metoclopramide, methotrimeprazine and haloperidol were powerful scavengers of hypochlorous acid. Haloperidol showed no ability to inhibit lipid peroxidation or to scavenge peroxyl radicals, and reproducibly increased lipid peroxidation catalysed by haem proteins, in both the presence and absence of hydrogen peroxide. The relevance of these *in vitro* observations to events *in vivo* is discussed.

Key words: neuroleptics; antioxidant; free radical; chlorpromazine; haem proteins; lipid peroxidation

Reactive oxygen species, such as superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), are known to be generated in the brain and nervous system *in vivo* [1–3]. In the presence of transition metal ions, especially iron ions, $O_2^{\cdot-}$ and H_2O_2 can be converted into highly damaging hydroxyl ($OH^{\cdot}$) radicals [4]. Iron ions also accelerate the free radical chain reaction of lipid peroxidation. Several areas of the human brain are rich in iron [5] which appears to be easily mobilizable in a form that can stimulate free radical reactions, such as $OH^{\cdot}$ formation and lipid peroxidation [5–7]. In addition, superoxide reacts with nitric oxide radical ($NO^{\cdot}$) to form the toxic species peroxynitrite, which can decompose to form several cytotoxic species, including $OH^{\cdot}$ [8]. Indeed, free radical damage has been suggested to play an important role in neurodegenerative diseases [1, 9, 10] and in the sequelae of ischaemic or traumatic CNS injury [6, 7, 11].

It has been further suggested that several neuroleptic drugs may be able to influence free radical reactions *in vivo* and that such reactions may contribute to their side-effects [12–14]. For example, haloperidol administration to rats increased lipid peroxidation and decreased levels of reduced glutathione (GSH) in the brain [15]. End-products of lipid peroxidation in the cerebrospinal fluid were claimed to be elevated in patients taking phenothiazines [16]. Chlorpromazine metabolites have been suggested to generate H_2O_2 by autoxidation [17, 18]. By contrast, some authors report that promethazine [19] and chlorpromazine [20, 21] *inhibit* lipid peroxidation *in vitro*, although there is a conflicting report that chlorpromazine can accelerate lipid peroxidation in the presence of light [20]. The ability of neuroleptics to interact with iron both *in vivo* and *in vitro* is well established [22, 23] and it would be of interest to understand how this could influence iron-dependent free radical reactions.

Pro-oxidant and antioxidant ability can be exerted in numerous ways *in vivo*, e.g. by alteration of endogeneous antioxidant defence enzymes and/or by a direct antioxidant or pro-oxidant ability of the compound itself. In order to clarify the direct ability of neuroleptics to inhibit or aggravate oxidative damage, we undertook a detailed characterization of their antioxidant and pro-oxidant ability *in vitro*. Biologically relevant reactive oxygen species and assays [24] were used. We report here the ability of neuroleptics to react with hydroxyl ($OH^{\cdot}$) and superoxide ($O_2^{\cdot-}$) radicals and to affect iron-dependent $OH^{\cdot}$ generation and lipid peroxidation. We also examined the reaction of neuroleptics with hypochlorous acid (HOCl), a damaging species produced by activated phagocytes [25], and their ability to affect lipid peroxidation caused by a mixture of a haem protein and H_2O_2. Haemoglobin and myoglobin can accelerate free radical damage in the presence of H_2O_2, and this action of haemoglobin may occur *in vivo* after brain haemorrhage [3, 26].

* Corresponding author.

MATERIALS AND METHODS

Chemicals were of the highest quality available and were purchased from Sigma Chemical Co. (Poole, U.K.) or from BDH Chemical Co. (Gillingham, U.K.), except for methotrimeprazine (May and Baker Ltd, Dagenham, U.K.) and metoclopramide (Beechams, U.K.).

Reactions with hypochlorous acid. Reaction with hypochlorous acid (HOCl) was studied using the elastase assay [27]. HOCl (60 μM, produced immediately before use by adjusting NaOCl to pH 6.2 with dilute H_2SO_4) and the compounds to be tested were incubated in a final volume of 1.0 mL in PBS, pH 7.4, containing 140 mM NaCl, 2.7 mM KCl, 16 mM Na_2HPO_4 and 2.9 mM KH_2PO_4 at 25°. To the reaction mixture, 0.2 mL of α_1-AP* (Sigma type A9024) (1.2 mg/mL) was added. This allows any HOCl remaining to inactivate α_1-AP. After 20 min further incubation, 0.05 mL of 1.0 mg/mL elastase (Sigma type EO258) was added. Any HOCl remaining is diluted out to the point at which it cannot affect elastase, by addition of 2 mL PBS. This mixture was allowed to stand for a further 30 min at 25° to allow any α_1-AP still active to inhibit elastase. The elastase activity remaining was measured by adding 0.1 mL of elastase substrate (5 mg/mL), *N*-succinyl-trialanyl-*p*-nitroanilide), and monitoring increases in absorbance at 410 nm.

Peroxidation of phospholipid liposomes. The ability of compounds to inhibit lipid peroxidation at pH 7.4 was tested using ox brain phospholipid liposomes [28]. Experiments were conducted in a physiological saline buffer (3.4 mM Na_2HPO_4-NaH_2PO_4, 0.15 M NaCl), pH 7.4. Assay mixtures contained, in a final volume of 1 mL made up with buffer, 0.5 mg/mL phospholipid liposomes, 100 μM $FeCl_3$, varying concentrations of the compounds dissolved either in water or in ethanol (ethanol does not affect the lipid peroxidation assay) and 100 μM ascorbate (added last to start the reaction). Incubations were at 37° for 60 min. At the end of this incubation period, 0.1 mL of 2% (w/v) BHT was added to each mixture followed by addition of 1 mL each of 1% (w/v) TBA and 2.8% (w/v) trichloroacetic acid. The solutions were heated in a water bath at 80° for 20 min to develop the $(TBA)_2$–MDA adduct. Addition of BHT to the reaction mixtures minimizes erroneous increases in colour due to iron ion-dependent hydroperoxide decomposition during the acid heating stage. The $(TBA)_2$–MDA chromogen was extracted into 2 mL butan-1-ol and the extent of peroxidation measured, in the organic layer, as absorbance at 532 nm.

Reactions with trichloromethyl peroxyl radicals. Reaction with trichloromethyl peroxyl radical was conducted using the Linear Accelerator Facility at the Paterson Institute, Christie Hospital (Manchester, U.K.). Reaction mixtures contained 1% (v/v) CCl_4 and 50% (v/v) isopropyl alcohol in 10 mM KH_2PO_4–KOH, pH 7.4, and 0.05% (w/v) of the compounds studied [29]. The absolute rate constants were calculated from the reaction kinetics [30, 31].

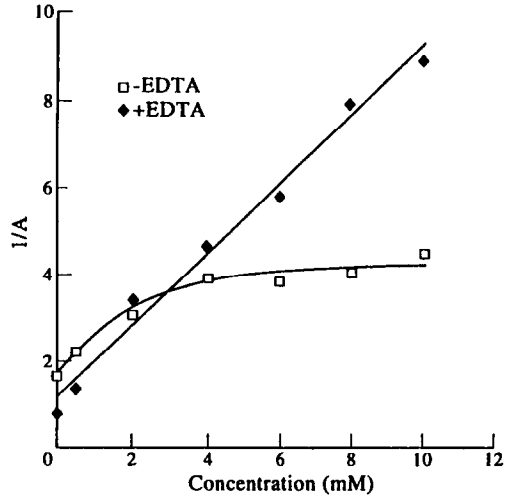

Fig. 1. Scavenging of hydroxyl radicals by chlorpromazine. Details of the reaction mixtures are given in Materials and Methods and in Ref. 34. The rate constant for reaction of chlorpromazine with $OH^{\cdot}$ was calculated from the ◆ graph as slope × k_{DR} × [DR] × A°, where k_{DR} is 3.1×10^9 M^{-1} sec^{-1}, A° is the absorbance in the absence of added chlorpromazine and [DR] is the concentration of deoxyribose in the reaction mixture.

Reaction with superoxide radical. Generation of $O_2^{\cdot-}$ was achieved using the hypoxanthine-xanthine oxidase system [32]. Reaction mixtures contained, in a final volume of 3 mL, 0.1 mL 30 mM hypoxanthine (dissolved in minimum 50 mM potassium hydroxide solution), 0.1 mL 0.3 mM EDTA, either 0.1 mL 3 mM cytochrome c or 0.1 mL 3 mM NBT and 88 mM (final concentration) KH_2PO_4–KOH buffer (pH 7.4). In each case, the reaction was started by adding 0.3 mL of xanthine oxidase (Sigma type X1875, freshly diluted in the above phosphate buffer to give appropriate enzyme activity per mL) and the rate of cytochrome c or NBT reduction measured at 550 or 560 nm, respectively, at 25°.

Bleomycin-dependent DNA damage. The bleomycin assay was carried out by a modification of the method of Gutteridge *et al.* [33]. Reaction mixtures contained, in a final volume of 1.0 mL, the following reagents at the final concentrations stated: DNA (0.2 mg/mL), bleomycin (0.05 mg/mL), $FeCl_3$ (0.025 mM), $MgCl_2$ (5 mM), KH_2PO_4–KOH buffer, pH 7.0 (30 mM), and ascorbate (where used, 0.24 mM) or the compounds to be tested, at varying concentrations. Compounds were either dissolved in water or in ethanol (control experiments showed that ethanol itself has no effect on the bleomycin assay). Reaction mixtures were incubated at 37° for 1 hr. At the end of the incubation period, 0.1 mL of 0.1 M EDTA was added to stop the reaction (the iron–EDTA complex is unreactive in the bleomycin assay). The coloration was obtained by adding 1 mL 1% (w/v) TBA and 1 mL 25% (v/v) HCl followed by heating in a water-bath maintained at 80° for

* Abbreviations: α_1-AP, α_1-antiproteinase; TBA, thiobarbituric acid; BHT, butylated hydroxytoluene; NBT, nitroblue tetrazolium; MDA, malondialdehyde; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Table 1. Rate constants for hydroxyl radical scavenging

Drug tested	Concentration range tested (mM)	Rate constant for $OH^{\cdot}$ scavenging $M^{-1} sec^{-1}$
Chlorpromazine	0–10	8×10^9
Prochlorperazine	0–2.5	5×10^9
Metoclopramide	0–6	3×10^9
Methotrimeprazine	0–0.65	3×10^{10}
Mannitol*	—	$(1.0–1.8) \times 10^9$
Ethanol*	—	$(0.7–1.1) \times 10^9$

Results are the means of two or three separate determinations whose results differed by ≤5%. Rate constants were calculated as described in the legend to Fig. 1 and in Ref. 34.

* Data abstracted from Ref. 34 for comparison.

15 min. The chromogen formed was extracted into butan-1-ol and the absorbance was measured at 532 nm.

Deoxyribose assay. The deoxyribose assay was carried out as described in Ref. 34. In some reaction mixtures, EDTA was omitted to test for iron ion binding-dependent inhibition of $OH^{\cdot}$ generation [35, 36].

Haem protein-dependent lipid peroxidation. Myoglobin or haemoglobin (50 μM haem), arachidonic acid (0.4 mM), the drugs at the various concentrations tested and (where indicated) H_2O_2 (0.5 mM) were incubated together for 10 min at 37° in KH_2PO_4–KOH buffer (50 mM, pH 7.4). The products of peroxidation were detected using the TBA test as described in Ref. [37]. All drugs were added in aqueous solution except for haloperidol which was dissolved in ethanol, which has no effect on the assay.

RESULTS

Scavenging of hydroxyl radicals

Hydroxyl radicals ($OH^{\cdot}$) were generated by a mixture of ascorbic acid, H_2O_2, and ferric-EDTA:

$$Fe^{3+}\text{-EDTA} + \text{ascorbate} \rightarrow Fe^{2+}\text{-EDTA} + \text{oxidized ascorbate} \quad (1)$$

$$Fe^{2+}\text{-EDTA} + H_2O_2 \rightarrow Fe^{3+}\text{-EDTA} + OH^{\cdot} + OH^{-} \quad (2)$$

They were detected by their ability to degrade the sugar deoxyribose into a product that reacts with thiobarbituric acid to form a chromogen. Any molecule that scavenges $OH^{\cdot}$ competes for $OH^{\cdot}$ with the deoxyribose and so decreases the rate of deoxyribose degradation. Figure 1 shows representative data for chlorpromazine, which competitively inhibited deoxyribose degradation by $OH^{\cdot}$. From the kinetics of the competition (see legend to Fig. 1 and Ref. 34), a rate constant of $8 \times 10^9 M^{-1} sec^{-1}$ for the reaction of chlorpromazine with $OH^{\cdot}$ was calculated. This high value shows that the reaction of chlorpromazine with $OH^{\cdot}$ is almost diffusion controlled. Table 1 summarizes the data for several other drugs and compares them with "established" $OH^{\cdot}$ radical scavengers such as mannitol and ethanol. Unfortunately, haloperidol could not be tested in this assay system since it is insoluble in water and organic solvents interfere with the deoxyribose assay [34].

Table 2. Effect of drugs in inhibiting iron ion-dependent generation of hydroxyl radicals from H_2O_2

Drug tested	Concentration (mM)	Inhibition of deoxyribose degradation (%)
Chlorpromazine	0	0
	0.10	36
	0.20	44
	0.40	48
	0.60	48
Prochlorperazine	0.10	24
	0.20	28
	0.40	29
	0.60	29
Metoclopramide	0.10	8
	0.20	14
	0.40	14
	0.60	21
Methotrimeprazine	0.10	27
	0.20	28
	0.50	38
	1.00	48

Drugs were tested at the final concentrations stated. Percentage values are the means of two or three determinations that varied by ≤5%.

Control experiments (listed in Ref. 24) showed that none of the drugs interfered with the assay system. Omission of ascorbate from the reaction mixtures always inhibited $OH^{\cdot}$ generation, suggesting that the drugs themselves cannot reduce the iron complex (equation 1) and lead to $OH^{\cdot}$ production.

Ability to inhibit iron ion-dependent hydroxyl radical generation

If iron is added to the deoxyribose assay as $FeCl_3$ instead of as ferric-EDTA, some of the iron ions bind to the deoxyribose so that $OH^{\cdot}$ is formed directly upon this molecule (rather than in free solution) and so scavengers of $OH^{\cdot}$ cannot inhibit deoxyribose degradation [35, 36]. The only molecules that can inhibit deoxyribose degradation under these reaction conditions are those that chelate iron ions from the deoxyribose and render them inactive in catalysing $OH^{\cdot}$ generation [35, 36]. Hence this version of the deoxyribose assay is a test for the ability of a compound to inhibit iron ion-dependent $OH^{\cdot}$ generation [24, 35, 36]. Table 2 and Fig. 1 (line in absence of EDTA) show that chlorpromazine exerted moderate antioxidant effects in this system, peaking at about 50% inhibition. This suggests that chlorpromazine can bind iron and partially inhibit $OH^{\cdot}$ generation. The other drugs tested were much less inhibitory (Table 2).

Inhibition of lipid peroxidation in liposomes

When ox brain phospholipid liposomes are

Table 3. Effect of drugs on liposomal lipid peroxidation

Drug tested	Concentration (mM)	Inhibition of peroxidation (%)
Chlorpromazine	0.005	4 ± 2
	0.025	20 ± 5
	0.050	39 ± 7
	0.1	60 ± 5
	0.25	79 ± 8
	0.5	87 ± 4
Prochlorperazine	0.005	4 ± 1
	0.025	28 ± 6
	0.050	52 ± 5
	0.1	70 ± 6
	0.25	83 ± 6
	0.5	89 ± 6
Methotrimeprazine	0.005	4 ± 2
	0.025	26 ± 3
	0.050	48 ± 6
	0.1	69 ± 9
	0.25	77 ± 7
	0.5	77 ± 8
Haloperidol	0.1	23 ± 9
Metoclopramide	0.1	8 ± 5

Results are means ± SD of three or more experiments. Concentrations are the final concentration in the reaction mixtures.

Table 4. Second-order rate constants for reaction of drugs with trichloromethylperoxyl radicals

Drug	Rate constant ($M^{-1}\,sec^{-1}$)
Metoclopramide	No reaction detected
Haloperidol	No reaction detected
Prochlorperazine	4×10^8
Promethazine	3×10^8
Chlorpromazine	3×10^8
Methotrimeprazine	4×10^8
Propyl gallate*	1.67×10^7
Trolox C*	2.23×10^8

Results are means of three or more determinations that varied by ≤5%.

* Values of these two established antioxidants are included for comparison. Data from Ref. 39.

incubated in the presence of $FeCl_3$ and ascorbate, they undergo rapid peroxidation, which can be measured by the TBA test. This experimental system offers an advantage over biological membranes (e.g. microsomes) or tissue homogenates in testing antioxidant ability, since the results are not affected by the presence of endogenous membrane antioxidants. Table 3 shows that chlorpromazine, methotrimeprazine and prochlorperazine are excellent inhibitors of lipid peroxidation, whereas haloperidol and the other drugs tested had much smaller effects. Control experiments showed that none of the drugs affected the measurement of lipid peroxidation.

Scavenging of peroxyl radicals

Inhibition of lipid peroxidation is often due to scavenging of peroxyl radicals, key intermediates in the chain reaction [24]. The ability of the drugs to do this was examined directly by generating a model organic peroxyl radical, trichloromethylperoxyl ($CCl_3O_2^{\cdot}$) by exposing a mixture of carbon tetrachloride (CCl_4), propan-2-ol and buffer to ionizing radiation, so producing hydrated electrons (e_{aq}^-) and $OH^{\cdot}$

$$e_{aq}^- + CCl_4 \rightarrow {}^{\cdot}CCl_3 + Cl^- \quad (3)$$

$$OH^{\cdot} + CH_3CHOHCH_3 \rightarrow H_2O + CH_3\dot{C}OHCH_3 \quad (4)$$

$$CH_3\dot{C}OHCH_3 + CCl_4 \rightarrow CH_3COCH_3 + {}^{\cdot}CCl_3 + H^+ \quad (5)$$

$$^{\cdot}CCl_3 + O_2 \rightarrow CCl_3O_2^{\cdot} \quad (6)$$

Promethazine has already been shown to react fast with trichloromethylperoxyl radical [38] and we confirmed this (Table 4). The data in Table 4 show that promethazine and prochlorperazine also react fast with $CCl_3O_2^{\cdot}$, whereas haloperidol had no detectable scavenging activity (rate constant $<10^5\,M^{-1}\,sec^{-1}$). Table 4 also shows data obtained with some established chain-breaking antioxidants for comparison.

Effect on haem protein-dependent lipid peroxidation

Mixing haem proteins with hydrogen peroxide generates powerfully oxidizing activated haem species and radicals on amino acid side-chains that can cause lipid peroxidation [40, 41]. As a model of such reactions, we used the peroxidation of arachidonic acid by a mixture of myoglobin and H_2O_2 [37], especially as it has already been reported that phenothiazines can be oxidized to radicals by activated myoglobin and other haem proteins [42, 43]. Table 5 shows the results obtained. Metoclopramide had no significant effect. Prochlorperazine, methotrimeprazine and chlorpromazine inhibited peroxidation, but only at high concentrations. By contrast, haloperidol reproducibly *increased* peroxidative damage to arachidonic acid, with a maximum effect being shown at 0.5 mM concentrations. Even when H_2O_2 was omitted, haloperidol stimulated peroxidation. Control experiments showed that haloperidol did not affect any of the assays being used to measure peroxidation. Very similar results were obtained using haemoglobin instead of myoglobin (Table 5).

Reaction with superoxide radical, $O_2^{\cdot-}$

A mixture of hypoxanthine and xanthine oxidase at pH 7.4 generates $O_2^{\cdot-}$, which can be measured by its ability to reduce ferricytochrome c to ferrocytochrome c, followed as a rise in absorbance at 550 nm [32]. An alternative measure of $O_2^{\cdot-}$ is the reduction of NBT to formazan, followed at 560 nm. These assays have been adapted to assess the ability of antioxidants to react with $O_2^{\cdot-}$ [24]. However, none of the drugs, tested at final concentrations up

Table 5. Effects of drugs on haem protein/H_2O_2-dependent peroxidation of arachidonic acid

Addition to assay	Drug concentration (mM)	Extent of peroxidation A_{532}
AA.MB	—	0.21
AA.MB.H_2O_2	—	0.47
AA.MB.H_2O_2 + methotrimeprazine	0.05	0.38
	0.2	0.33
	0.5	0.20
	1.0	0.21
AA.MB.H_2O_2 + metoclopramide	0.05	0.51
	0.2	0.45
	0.5	0.43
	1.0	0.42
AA.MB.H_2O_2 + chlorpromazine	0.05	0.42
	0.2	0.48
	0.5	0.33
	1.0	0.28
AA.MB.H_2O_2 + prochlorperazine	0.05	0.38
	0.2	0.37
	0.5	0.24
	1.0	0.22
AA.MB.H_2O_2 + haloperidol	0.05	0.48
	0.2	0.53
	0.5	0.87
	1.0	0.74
AA.HB.H_2O_2 + haloperidol	0	0.42
	0.1	0.40
	0.25	0.42
	0.5	0.52
	0.75	0.45
AA.MB + haloperidol	0.05	0.25
	0.2	0.33
	0.5	0.70
	1.0	0.74
AA.HB + haloperidol	0	0.16
	0.1	0.17
	0.25	0.21
	0.50	0.47
	0.75	0.37

Peroxidation was measured by the TBA test and the results are presented as absorbance at 532 nm. Drugs were added to give the final concentrations stated. The table lists the means of at least 4 assays which had a variation in absorbance of <5%. MB—myoglobin; HB—haemoglobin; AA—arachidonic acid.

to 0.5 mM, showed any significant ability to scavenge O_2^- (data not shown).

Bleomycin test for pro-oxidant activity

Several compounds that chelate iron ions can sometimes exert pro-oxidant effects in systems involving iron-dependent free radical damage [24, 29, 44]. One way of testing for such pro-oxidant activity is to examine the ability of compounds to accelerate damage to DNA in the presence of ferric bleomycin [24, 44]. However, chlorpromazine, prochlorperazine, metoclopramide, methotrimeprazine or haloperidol, tested at concentrations up to 0.5 mM, had no pro-oxidant activity in this assay system.

Scavenging of hypochlorous acid

Hypochlorous acid (HOCl) is a powerful oxidizing and chlorinating agent generated by activated phagocytes [25]. It damages several biological targets, e.g. it is a powerful inhibitor of α_1-AP, an important inhibitor of serine proteases (such as elastase) in human body fluids [25]. Hence an established assay for the ability of a compound to scavenge HOCl is to test its action in preventing inactivation of α_1-AP by HOCl [24, 27]. Table 6 shows that all the drugs tested were excellent scavengers of HOCl, able to protect α_1-AP almost fully even at only 100 μM concentrations (when 60 μM HOCl was present)

DISCUSSION

In order to begin to evaluate suggestions in the literature that neuroleptic drugs may exert antioxidant and/or pro-oxidant actions *in vivo*, we

Table 6. Scavenging of hypochlorous acid by drugs

Reaction mixture	Elastase activity (%)	α_1-Antiproteinase activity (%)
Elastase only	100	0
Elastase plus α_1AP	0.1	99.9
Elastase plus α_1AP + HOCl	100	0
plus chlorpromazine	0.2	99.8
plus prochlorperazine	0.9	99.1
plus metoclopramide	0.3	99.7
plus methotrimeprazine	0.7	99.3
plus haloperidol	0.4	99.6

α_1-Antiproteinase (α_1AP) inhibits the enzyme elastase. HOCl inactivates α_1-antiproteinase, so that elastase activity is restored. A good scavenger of HOCl protects the α_1-antiproteinase, so that elastase remains inhibited. Control experiments showed that none of the drugs tested affected elastase or α_1-antiproteinase directly. Drugs were present at a final concentration of 100 μM in the reaction mixtures. Results are the means of at least three experiments that differed by $\leq$10%.

examined their ability to exert direct antioxidant and pro-oxidant actions *in vitro*, using a well-established battery of tests [24]. None of the drugs scavenged $O_2^{\cdot-}$. By contrast, all the drugs could scavenge highly reactive $OH^{\cdot}$ radicals and hypochlorous acid at rates comparable to "established" free radical scavengers. In addition, chlorpromazine showed some ability to bind iron ions and inhibit $OH^{\cdot}$ generation, presumably by rendering the iron ions unreactive with H_2O_2 [24, 36]. This drug, prochlorperazine, promethazine and methotrimeprazine, were also powerful scavengers of an organic peroxyl radical ($CCl_3O_2^{\cdot}$), indicative of their ability to act as chain-breaking antioxidants. Indeed, they were powerful inhibitors of liposomal lipid peroxidation stimulated by iron and ascorbate. They also inhibited, at somewhat higher concentrations, arachidonic acid peroxidation stimulated by haem proteins plus H_2O_2. Thus, although haem proteins can oxidize some of these drugs into radicals, these radicals seem unable (or at least less able than the haem proteins themselves) to initiate lipid peroxidation. The report of Pall *et al.* [12] that phenothiazines accelerate lipid peroxidation *in vivo* seems unlikely therefore to be explained by a *direct* pro-oxidant effect of these compounds.

In view of claims that haloperidol might be metabolized in a way similar to MPTP [45] and cause oxidative damage *in vivo* [15], it was of particular interest to note that this drug significantly accelerates arachidonic acid peroxidation by haem proteins in both the presence and absence of H_2O_2 (Table 5). This ability to stimulate peroxidation has already been observed for the anti-inflammatory drug phenylbutazone, which produces numerous side-effects that have greatly restricted its clinical use [37]. The mechanism of this effect is being investigated. We believe that haloperidol is oxidized by haem proteins to a highly reactive radical that initiates lipid peroxidation.

We emphasize that our results are obtained *in vitro* only. The value of these tests is that they enable one to investigate the possibility of *direct* pro-oxidant or antioxidant effects of compounds *in vivo*. The fact that such effects could be feasible (as shown by *in vitro* testing) does not mean that they actually occur *in vivo*, and further studies are underway to examine this question. Another important question is whether the concentrations at which the drugs exert antioxidant effects are relevant to the concentrations present *in vivo*. The ability of lipophilic drugs to concentrate within hydrophobic regions, such as the interior of membranes, must not be ignored.

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