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CHLORPROMAZINE:

FOR BETTER FOR WORSE

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Abbreviations:

a_1-AP – antiproteinase, AP-aminopyrine, CPZ- chlorpromazine, Cys- cysteine, DMPO- 5,5-dimethyl-pyroline-N-oxide, DMSO- dimethyl sulfoxide, FMP-formyl-methionyl-leucyl-phenylalanine, GLA- γ-linolenic acid, GSH- glutathione, HOCl- hypochlorous acid, HRP-horseradish peroxidase, LPO- lipoperoxidase, LPS- lipopolisaccharide, MPO- myeloperoxidase, MNP- 2-methyl-2-nitrosopropane, NADH- reduced nicotinamide- adenine dinucleotide, PMA-phorbol myristate acetate, PZ- promazine, TBA- thiobarbituric acid, TNF- tumor necrosis factor, TFP-trifluoperazine

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1. Abstract

Chlorpromazine (2-chloro-N, N-dimethyl-10H-phenothiazine-10-propanamine) is a neuroleptic drug, used for the lifelong treatment of severe psychoses such as schizophrenia and mania. Its side effects are often serious and unpredictable. Attempts to better understand and prevent those side effects have included the study of the complex biochemical pharmacology of CPZ, focusing on its actions *via* its cation radical, $CPZ^{\bullet+}$, which is released during the photoionization of CPZ under UV irradiation, as well as during several oxidation processes following the metabolism of the drug in the human body. On the other hand, CPZ often acts as an antioxidant and free radical scavenger (e.g., in human leukocytes and liposomes). This review paper addresses the balance between these two antagonistic actions.

2. Introduction

Antipsychotic drugs are used to treat psychoses, the most severe psychiatric disorders, such as schizophrenia and mania. Psychoses are characterized by a severe form of disordered thinking and relating, resulting from disturbances in reality perception. The most frequent psychosis is schizophrenia, which afflicts 1% of the world population. It is a life-long, progressive illness with onset at the time of adolescence or young adulthood, manifested by the so-called "positive symptoms" (hallucinations, agitation, delusions of persecution, disordered thinking, bizarre

motor behavior, and suspiciousness) as well as by "negative symptoms" (emotional apathy, poverty of speech, extreme inattentiveness and social withdrawal). Psychoses are believed to be caused by the excessive activity of dopaminergic neurons or excess of specific subtype of dopamine receptors, and virtually all antipsychotic drugs act by blocking dopaminergic receptors. In the case of schizophrenia, antipsychotic drugs best effect the positive symptoms and improve mood and behavior in 50% of cases; the rest are refractory to treatment [1].

Based on their chemical structure, antipsychotic drugs (also called neuroleptics) are classified into the following categories: phenothiazines, thioxanthenes, butyrophenones, dibenzoxazepines, dihydroindolones, dibenzodiazepines, and bensizoxasoles. Phenothiazines are further classified into three subclasses: aliphatic (*e.g.* chlorpromazine), piperidine (*e.g.* thioridazine) and piperazine (*e.g.* fluphenazine) [1].

The structure of chlorpromazine is shown in Figure 1 (from [1]):

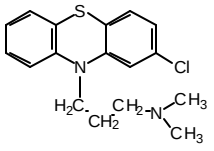


Fig.1. The chemical structure of chlorpromazine

The daily dose of CPZ is 300-900 mg. Its side effects include sedation, drowsiness, orthostatic hypotension resulting in syncope, reflex tachycardia, ocular toxicity *(e.g.* lenticular deposits), Parkinson-like syndromes, tardive dyskinesia (involuntary facial movements), blood dyscrasias (*e.g.* agranulocytosis), cholestatic jaundice, dermatitis due to the photosensitivity of skin exposed to sunlight, and impotence or infertility [1].

1. Chlorpromazine photoionization

The photoallergic and phototoxic reactions of CPZ in humans, as well as its capacity of inducing photomutagenesis in bacteria, have been attributed to the formation of free radicals. Under UV irradiation, CPZ photoionizes in aqueous solution to produce the cation radical and

the hydrated electron by a stepwise biphotonic mechanism for photoionization of CPZ *via* a triplet excited state of CPZ [2]:

$$CPZ \xrightarrow{h\nu} CPZ^* \quad \text{(initial excitation)} \quad (1)$$

$$^1CPZ^* \rightarrow {}^3CPZ^* \quad \text{(intersystem crossing)} \quad (2)$$

$$^3CPZ^* \xrightarrow{h\nu} CPZ^{2+} + e_{aq}^- \quad \text{(secondary excitation)} \quad (3)$$

The hydrolysis of the cation radical leads to a phenothiazine sulfoxide [3]:

$$CPZ^{\bullet+} + H_2O \rightarrow CPZ\text{-}SO + e^- + 2H^+ \quad (4)$$

$$CPZ\text{-}SO + H_2O \rightarrow CPZ\text{-}SO_2 + 2e^- + 2H^+ \quad (5)$$

In the eye, metabolically formed sulfoxide may itself be an active pharmacologic agent which is involved in lens damage. This is significant, since over 50% of the CPZ taken orally is metabolized to CPZ-SO [3]. The photolysis of the sulfoxide, *via* the homolytic cleavage of the S=O bond of the sulfoxide, generates a hydroxyl free radical that is capable of oxidizing ascorbate, Cys, GSH, NADH, azide, DMSO and ethanol. The cleavage takes place after excited-state protonation of the sulfoxide to a form which then yields CPZ^{2+} and $^{?}OH$:

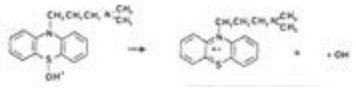
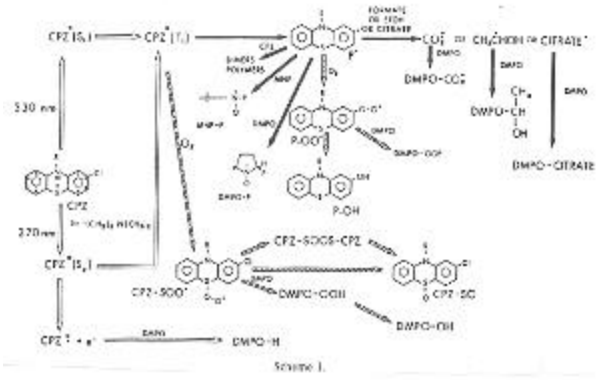


Fig. 2. Formation of the free hydroxyl radical (from [3])

Of significant biological importance, since dechlorinated metabolites of CPZ were found in high concentrations in the body of psychotic patients [4], is the process of CPZ dechlorination to form a PZ radical that is able to extract a hydrogen atom from a variety of donors such as ethanol, citrate and formate to yield PZ, a known photoproduct of CPZ. The PZ radical may react with another PZ radical or with CPZ to give dimers and higher polymers. All of the radical intermediates mentioned above will form further photoproducts, as outlined in Scheme 1(from [5]), some which may also be radical intermediates. Figure 3, from [3], shows the flash photolysis of CPZ sulfoxide:



Scheme 1.

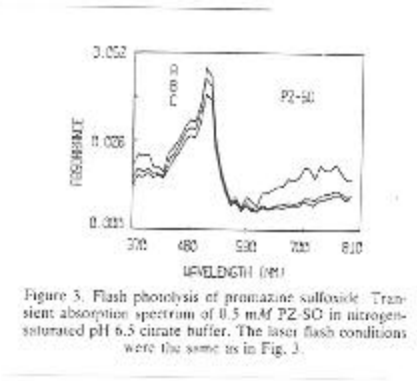


Figure 3. Flash photolysis of promazine sulfoxide. Transient absorption spectrum of 0.5 mM PZ-SO in nitrogen-saturated pH 6.5 citrate buffer. The laser flash conditions were the same as in Fig. 3.

The spin traps MNP and DMPO were used to define the radical photolysis of CPZ and PZ. In the absence of O_2, the dechlorination product of CPZ is trapped by MNP. The reactivity of the dechlorination product is similar to that of the phenyl radical as shown by its ability to extract H atoms from donors [5].

4. Chlorpromazine as a free radical scavenger

a) Scavenging of hydroxyl radicals

These radicals were generated by a mixture of ascorbic acid, H_2O_2, and ferric-EDTA:

$$Fe^{3+}\text{-EDTA} + \text{ascorbate} \rightarrow Fe^{2+}\text{-EDTA} + \text{oxidized ascorbate} \quad (6)$$

$$Fe^{2+}\text{-EDTA} + H_2O_2 \rightarrow Fe^{3+}\text{-EDTA} + {}^{?}OH + OH^{-} \quad (7)$$

Hydroxyl radicals were detected by their ability to degrade a sugar, deoxyribose, into a product that reacts with TBA to form a chromogen. Any molecule that scavenges $^{?}OH$ competes for OH^{-} with the deoxyribose and so decreases the rate of deoxyribose degradation [6]. Table 1 (from [6]) shows representative data for chlorpromazine compared to other drugs and "established" $^{?}OH$ radical scavengers such as mannitol and ethanol. As shown, CPZ competitively inhibited deoxyribose degradation by $^{?}OH$. The high value of the rate constant ($8 \times 10^9\ M^{-1}\ s^{-1}$) shows that the reaction of CPZ with $^{?}OH$ is almost diffusion-controlled. Another version of the deoxyribose assay showed that CPZ had antioxidant effects, by binding iron and partially inhibiting the generation of $^{?}OH$ radicals from H_2O_2 [6].

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*	—	$(0.7–1.1) \times 10^9$

b) Inhibition of lipid peroxidation in liposomes and scavenging of peroxyl radicals

When ox brain phospholipid liposomes were incubated in the presence of $FeCl_3$ and ascorbate, they underwent 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. It was found that CPZ is an excellent inhibitor of lipid peroxidation [6]. Inhibition of lipid peroxidation is often due to scavenging of peroxyl radicals, key intermediates in the chain reaction. The ability of the drugs to act in this capacity was examined by generating a model organic peroxyl radical, $CCl_3O_2^{?}$, by exposing a mixture of CCl_4, propan-2-ol and buffer to ionizing radiation and thus producing hydrated electrons (e_{aq}^-) and $^{?}OH$:

$$e_{aq}^- + CCl_4 \rightarrow {}^{?}CCl_3 + Cl^- \quad (8)$$

$$^{?}OH + CH_3CHOHCH_3 \rightarrow H_2O + CH_3COHCH_3 \quad (9)$$

$$CH_3COHCH_3 + CCl_4 \rightarrow CH_3COCH_3 + {}^{?}CCl_3 + H^+ \quad (10)$$

$$^{?}CCl_3 + O_2 \rightarrow CCl_3O_2^{?} \quad (11)$$

Data in Table 2 (from [6]) (drugs by comparison with some established chain-breaking antioxidants) show that some neuroleptic drugs, CPZ included, react fast with $CCl_3O_2^{?}$

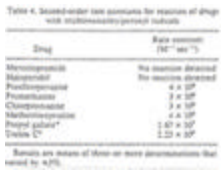
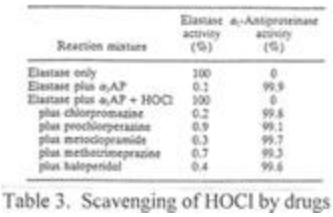


Table 2. Second-order rate constants for reaction of drugs with $CCl_3O_2^{\bullet}$

c) Scavenging of hypochlorous acid

HOCl is a powerful oxidizing and chlorinating agent generated by activated phagocytes. It damages several biological targets, *i.e.* it is a powerful inhibitor of α_1–AP, which in turn is an important inhibitor of serine proteases (such as elastase) in human body fluids. 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 [6]. Table 3 (from [6]) shows that CPZ and other neouroleptic drugs were excellent scavengers of HOCl, able to protect α_1-AP almost fully:



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

Table 3. Scavenging of HOCl by drugs

Table 3. Scavenging of HOCl by drugs

d) Bleomycin test for pro-oxidant activity

Some compounds that chelate iron ions (as CPZ was shown to do) can sometimes exert pro-oxidant effects in systems involving iron-dependent free radical damage. 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. However, most neuroleptics, CPZ included, all tested at up to 0.5 μM, had no pro-oxidant activity [6].

e) CPZ as a redox mediator in peroxidase-catalyzed oxidations

Peroxidases are heme-containing enzymes that are known to catalyze the one-electron oxidation of a variety of structurally diverse organic compounds using hydrogen peroxide as the oxidant in the peroxidase catalytic cycle [7]. Ferric peroxidase is oxidized by two electrons by hydrogen peroxide to yield water and compound I, an oxyferryl porphyrin p cation radical ($P^{?+}$) (12). Compound I accepts one electron from a reducing substrate, yielding compound II, or

oxyferryl heme, and one equivalent of substrate free radical (13). Compound II oxidizes an additional equivalent of substrate, generating ferric enzyme and the corresponding substrate free radical (14):

$$Fe(III) + H_2O_2 \; ? \quad Fe(IV)=O[P^{?+}] + H_2O \qquad (12)$$

$$Fe(IV)=O[P^{?+}] + RH_2 \; ? \quad Fe(IV)=O + RH^{?} \qquad (13)$$

$$Fe(IV)=O + RH_2 \; ? \quad Fe(III) + RH^{?} + H_2O \qquad (14)$$

Thus, the heme-containing peroxidases are known to catalyze the one-electron oxidation of a wide range of structurally diverse aromatic compounds [7]. One important reaction of radicals derived from peroxidases is that of redox mediation or mediation by electron transfer. The mechanism proposes that radicals ($A^{?+}$) generated by a peroxidase may act as diffusible oxidants to oxidize secondary molecules:

$$A^{?+} + B \; ? \quad A + B^{?+} \qquad (15)$$

Thus, the rate of generation of secondary radicals ($B^{?+}$) can be dramatically stimulated in the presence of a redox mediator (A). This type of mechanism may have toxicological implications if $B^{?+}$ or a subsequent metabolite is toxic. The presence of A may stimulate the production of toxic metabolites produced by peroxidases. One reaction catalyzed by peroxidases that may be affected by redox mediation is the oxidation of AP to its radical $AP^{?+}$. AP has been implicated as a cause of agranulocytosis [7]. CPZ, a common peroxidase substrate, was used as a redox mediator in a model system that included HRP and AP. It was demonstrated that $CPZ^{?+}$, generated by HRP, can oxidize AP to $AP^{?+}$ and that CPZ stimulates the rate of AP oxidation up to 1000-fold; it had a similar effect with LPO and MPO. Thus, redox mediation, similar to that observed between CPZ and AP, may play an important role in the toxicity of some medications or xenobiotics [7]. The same conclusion was reached independently [8] when it was shown that the peroxidase-catalyzed oxidation of hydrazines (drugs used for depression, hypertension, and

tuberculosis) may be significantly enhanced by the presence of efficient substrates acting as redox mediators such as CPZ. The addition of CPZ to a reaction mixture containing hydralazine (an aromatic hydrazine derivative), HRP and hydrogen peroxide significantly enhanced DNA strand breakage, as represented by decreased DNA migration upon electrophoresis in an agarose gel. Both the rate and the extent of peroxidase-catalyzed oxidation of hydralazine were increased in the presence of CPZ, which acted by significantly decreasing enzyme inactivation [8]. This process was mediated *via* the oxidation of CPZ to CPZ $^{\bullet+}$, which was monitored using UV-visible spectrophotometry. The oxidation of CPZ to $CPZ^{?+}$ by HRP -hydrogen peroxide is coupled to a non-enzymatic reaction causing the breakdown of the cation radical, subject to the nucleophilic attack of such species as $RCOO^-$, by the following mechanism [9]:

$$2\ CPZ + H_2O_2\ ?\quad 2\ CPZ^{?+} + 2\ H_2O \qquad (16)$$

$$CPZ^{?+} + RCOO^-\ ?\quad CPZ^{?}\text{-}OCOR \qquad (17)$$

$$CPZ^{?}\text{-}OCOR + CPZ^{?+}\ ?\quad CPZ^{+}\text{-}OCOR + CPZ \qquad (18)$$

$$CPZ^{+}\text{-}OCOR + H_2O\ ?\quad CPZ(OCOR)OH + H^+ \qquad (19)$$

$$CPZ(OCOR)OH\ ?\quad CPZ\text{-sulfoxide} + R\text{-}COOH \qquad (20)$$

f) Inhibition of free radical generation in human leukocytes

The respiratory burst of leukocytes during phagocytosis is accompanied by the release of free radicals. Several stimulants, *i.e.* PMA, FMP, LPS and TNF, are able to induce the respiratory burst in leukocytes. All induced free radical generation in human leukocytes is calmodulin-dependent and can be inhibited by calmodulin antagonists, which include CPZ and TFP [10]. Similarly, CPZ and TFP were shown to block free radical generation and lipid peroxidation process in HeLa cells stimulated by GLA and other fatty acids with cytotoxic action [11].

5. Summary

CPZ is a drug used for the lifelong treatment of schizophrenia. Its side effects are serious, often debilitating; some (photosensitivity, agranulocytosis) have been linked with the release of the free radical $CPZ^{?+}$. But CPZ is also able to act as an antioxidant (scavenger of HOCl and hydroxyl radicals, inhibitor of lipid peroxidation in liposomes and free radical generation in human leukocytes). The duality of the drug, as well as its impact on an already fragile health equilibrium, is studied in order to avoid any further impairment of the patients' quality of life.

6. References

1. Jacob LS. (1992) Agents acting on the central nervous system. In: Jacob LS, ed. *Pharmacology*. Malvern, PA: Harwal Publishing Company; pp. 45-79.
2. Hall RD, Buettner GR, Chignell CF. (1991) The biphotonic photoionization of chlorpromazine during conventional flash photolysis: spin trapping results with 5,5-dimethyl-1-pyrolline-*N*-oxide. *Photochem Photobiol* **54**:167-173.
3. Buettner GR, Motten AG, Hall RD, Chignell CF. (1986) Free radical production by chlorpromazine sulfoxide, an ESR spin-trapping and flash photolysis study. *Photochem Photobiol* **44**:5-10.
4. Valoti M, Palmi M, Della Corte L, Nardini M, Corti P, Sgaragli GP. (1992) Dehalogenation and N-dealkylation of chlorpromazine as revealed by plasma concentrations of metabolites in a population of chronically medicated schizophrenics. *Methods & Findings in Experimental and Clinical Pharmacology* **14**:445-450.
5. Motten AG, Buettner GR, Chignell CF. (1985) Spectroscopic studies of cutaneous photosensitizing agents - VIII. A spin-trapping study of light-induced free radicals from chlorpromazine and promazine. *Photochem Photobiol* **42**:9-15.
6. Jeding I, Evans PJ, Akanmu D, Dexter D, Spencer JD, Aruoma OI, Jenner P, Halliwell B. (1995) Characterization of the potential antioxidant and pro-oxidant actions of some neuroleptic drugs. *Biochem Pharmacol* **49**:359-365.
7. Goodwin Dc, Grover TA, Aust SD. (1996) Redox mediation in the peroxidase-catalyzed oxidation of aminopyrine: possible implications for drug-drug interactions. *Chem Res Toxicol* **9**:476-483.
8. Reilly CA, Aust SD. (1997) Peroxidase substrates stimulate the oxidation of hydralazine to metabolites which cause single-stranded breaks in DNA. *Chem Res Toxicol* **10**:328-334.
9. Vazquez A, Tudela J, Varon R, Garcia-Canovas F. (1992) A kinetic study of the generation and decomposition of some phenothiazine free radicals formed during enzymatic oxidation of phenothiazines by peroxidase-hydrogen peroxide. *Biochem Pharmacol* **44**:889-894.
10. Das UN, Padma M, Sagar PS, Ramesh G, Koratkar R. (1990) Stimulation of free radical generation in human leukocytes by various agents including tumor necrosis factor is a calmodulin-dependent process. *Biochem Biophys Res Commun* **167**:1030-1036.
11. Sagar PS, Das UN, Koratkar R, Ramesh G, Padma M, Kumar GS. (1992) Cytotoxic action of cis-unsaturated fatty acids on HeLa cell: relationship to free radicals and lipid peroxidation. *Cancer Letters* **63**:189-198.