

Photoinduced Free Radicals from Chlorpromazine and Related Phenothiazines: Relationship to Phenothiazine-Induced Photosensitization

by Colin F. Chignell,* Ann G. Motten,* and Garry R. Buettner*

Chlorpromazine and several other related phenothiazines are known to cause both phototoxic and photoallergic reactions in the skin and eyes of patients receiving these drugs. While the detailed mechanisms of photosensitization are not known, it is obvious that the first step must be the absorption of light by the drug, its metabolites, or photoproducts, or possibly an induced endogenous chemical. In this review, the free-radical photochemistry of phenothiazines is described, and the evidence for the involvement of photoinduced free radicals in photosensitization is examined. Upon irradiation chlorpromazine yields a variety of free radicals including the corresponding cation radical (via photoionization), the neutral promazinyl radical and a chlorine atom (Cl^{\bullet}) (via homolytic cleavage), and a sulfur-centered peroxy radical. The chlorpromazine cation radical is probably responsible for some of the observed *in vitro* phototoxic effects of this drug. However, it seems unlikely that the cation radical is involved in phototoxicity *in vivo*, since photoionization only occurs when chlorpromazine is excited into the S_2 level ($\lambda_{ex} < 280$ nm). The promazinyl radical is a more likely candidate for the phototoxic species both *in vivo* and *in vitro*. In addition, this radical can react covalently with proteins and other macromolecules to yield antigens which could be responsible for the photoallergic response to chlorpromazine. Neither oxygen-derived radicals nor singlet oxygen ($^1O_2^*$), appear to be important in chlorpromazine photosensitization. In contrast, it would seem that promazine-induced phototoxicity may result in part from the generation of superoxide ($O_2^{\bullet-}$). The inability of promazine, which lacks a chlorine atom at the 2-position, to undergo homolytic fission to give the promazinyl radical, probably explains why this drug is much less phototoxic than chlorpromazine both *in vivo* and *in vitro*.

Introduction

The interaction of light with chemical agents present in the skin and eyes often results in the photosensitization of both human and animal subjects (1). The photosensitizing chemical may be endogenous (protoporphyrin), a drug (declomycin, sulfonamide), a topical agent (4-aminobenzoic acid and its esters in sunscreens) or an environmental agent (anthracene in coal tar) (2,3). Photosensitization may take the form of phototoxicity or photoallergy. The phototoxic response is essentially an exaggerated sunburn reaction (1,2), while photoallergy is a delayed hypersensitivity reaction (1,3). Although the detailed mechanisms of photosensitization

are not known, it is obvious that the first step must be the absorption of light by the chemical, its metabolites, photoproducts, or possibly an induced endogenous chemical.

The phenothiazine tranquilizers, e.g., chlorpromazine, have been used to treat many psychotic disorders, particularly those which involve hyperactivity and anxious excitement. However, chlorpromazine and several other related phenothiazines are known to cause both phototoxic and photoallergic reactions in patients receiving low doses of these drugs (4-6). High dosage and prolonged treatment can produce severe dermatitis that is frequently accompanied by darkening of the skin due to the deposition of melanin in lower layers of the dermis (5). Such patients may also suffer retinal damage, ocular opacity and loss of vision.

In this review, the free-radical photochemistry of phenothiazines will be described, and the evidence for

*Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

the involvement of photoinduced free radicals in phenothiazine photosensitivity will be examined. The structures of the phenothiazine drugs are given in Table 1.

Free-Radical Photoproducts from Phenothiazines

Carbon-Centered Radicals

Grant and Green have reported that, in aqueous so-

lution, chlorpromazine (CIP) is converted into promazine (PH) and 2-hydroxypromazine (POH) upon exposure to sunlight (?). These workers proposed that the chlorpromazine triplet underwent direct homolytic fission to yield a chlorine atom (Cl[•]) and the neutral promazinyl radical (P[•]), which reacted with the solvent to give the observed products

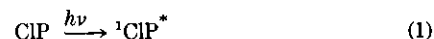
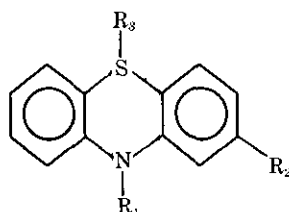
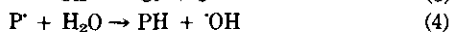


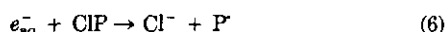
Table 1. Structures of the phenothiazines.

| Compound | R ₁ | R ₂ | R ₃ | Abbreviation |
|-----------------------------------|--|---|------------------|-------------------------------------|
| Phenothiazine | H | H | — | |
| Promazine | —(CH ₂) ₃ N(CH ₃) ₂ | H | — | PH |
| Chlorpromazine | —(CH ₂) ₃ N(CH ₃) ₂ | Cl | — | CIP |
| Triflupromazine | —(CH ₂) ₃ N(CH ₃) ₂ | CF ₃ | — | |
| Methoxypropazine | —(CH ₂) ₃ N(CH ₃) ₂ | OCH ₃ | — | POCH ₃ |
| Acepromazine | —(CH ₂) ₃ N(CH ₃) ₂ | COCH ₃ | — | |
| Compazine | —(CH ₂) ₃ -N(CH ₃) | Cl | — | |
| Perphenazine | —(CH ₂) ₃ -N(CH ₂ OH) | Cl | — | |
| Stelazine | —(CH ₂) ₃ -N(CH ₃) | CF ₃ | — | |
| Fluphenazine | —(CH ₂) ₃ -N(CH ₂ OH) | CF ₃ | — | |
| Promethazine | —CH ₂ CHN(CH ₃) ₂ CH ₃ | H | — | |
| Thioridazine | (CH ₂) ₂ -N(CH ₃) | SCH ₃ | — | |
| Trifluoperazine | (CH ₂) ₃ -N(CH ₃) | CF ₃ | — | |
| Chlorpromazine-MNP spin adduct | —(CH ₂) ₃ N(CH ₃) ₂ | —N—C(CH ₃) ₃ O | — | I |
| 2-Ethoxypropazine | —(CH ₂) ₃ N(CH ₃) ₂ | —OC ₂ H ₅ | — | POC ₂ H ₅ |
| 2-Isopropoxypropazine | —(CH ₂) ₃ N(CH ₃) ₂ | —OCH(CH ₃) ₂ | — | POCH(CH ₃) ₂ |
| 2-Dimethylaminopromazine | —(CH ₂) ₃ N(CH ₃) ₂ | —N(CH ₃) ₂ | — | PN(CH ₃) ₂ |
| 2-Hydroxypromazine | —(CH ₂) ₃ N(CH ₃) ₂ | OH | — | POH |
| 9-Peroxychlorpromazine | —(CH ₂) ₃ N(CH ₃) ₂ | Cl | —OO [•] | CIPSOO [•] |
| Chlorpromazine sulfoxide | —(CH ₂) ₃ N(CH ₃) ₂ | Cl | = O | CIPSO |





Moore and Tamat have reported that photolysis of chlorpromazine in nitrogen-saturated water results in the production of one mole of Cl^- per mole of drug photolyzed (8). The chloride ion may be derived from Cl^- by hydrogen abstraction from the solvent or possibly by reaction of electrons, derived from photoionization (*vide infra*), with ground-state chlorpromazine,



Recent spin-trapping studies have provided additional evidence that anaerobic photolysis of chlorpromazine at 330 nm results in the dechlorination to give the neutral radical, P^\cdot [Eq. (3)] (9). When 2-methyl-2-nitrosopropane (MNP) was used as a trap, one carbon-centered adduct (I) (Table 1) was detected from CIP over the range pH 3.5 to 6.5 (Fig. 1). The hyperfine splitting constants ($a^{\text{N}} = 14.1$ G; $a^{\text{H}} = 0.92$ G, and 1.99 G) of the adduct (I) were consistent with a structure containing three aromatic ring hydrogens derived from the reaction of MNP with the neutral promazine radical, P^\cdot . The detection of this spin adduct implies that P^\cdot is sufficiently stable to make the extraction of H^\cdot from water [Eq. (4)] and subsequent $\cdot\text{OH}$ formation very unlikely. P^\cdot is, however, able to extract H^\cdot from donors such as ethanol or citrate (9) to form promazine, PH, and could also react with molecular oxygen, ultimately forming POH (9).

In oxygen-free isopropanol, chlorpromazine also undergoes homolytic carbon-chlorine bond fission to form the neutral promazine radical (P^\cdot) which then

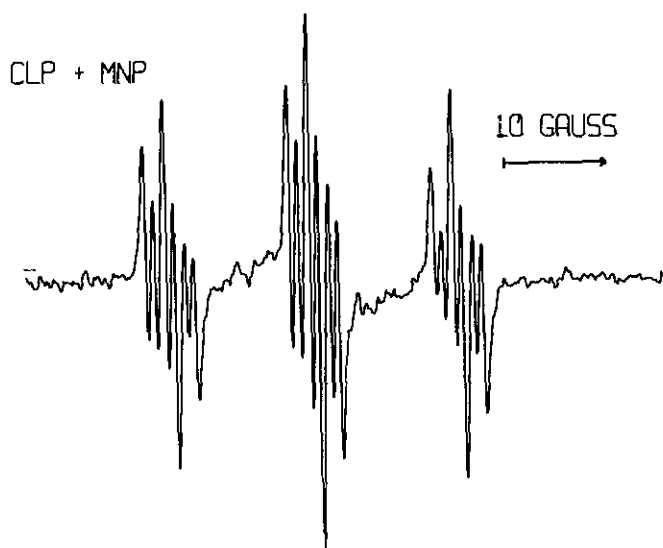
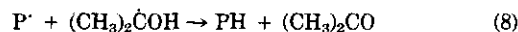


FIGURE 1. Electron spin resonance spectrum of adduct I obtained by irradiation of an aqueous solution (pH 4.0) of chlorpromazine and 2-methyl-2-nitrosopropane (MNP) at 330 nm.

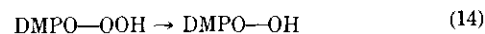
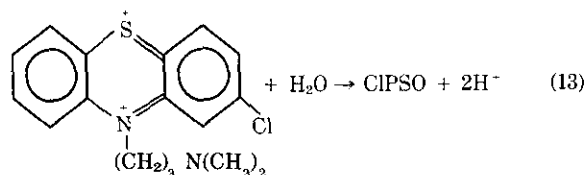
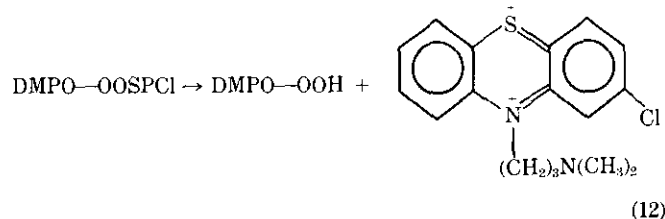
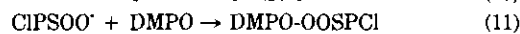
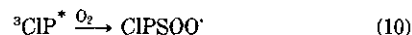
reacts with the solvent to form promazine, 2-isopropoxypromazine, HCl, and acetone (10):



A similar mechanism can be proposed to explain the production of promazine and other 2-substituted promazines when chlorpromazine is irradiated in other solvents, e.g., methanol (POCH_3), ethanol (POC_2H_5), and aqueous dimethylamine [$\text{PN}(\text{CH}_3)_2$] (7).

Oxygen-Centered Radicals

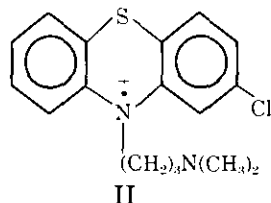
When an aqueous aerated solution of chlorpromazine is irradiated at 330 nm a peroxy radical is trapped by 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) which decays to the hydroxyl radical adduct, DMPO-OH (9). Since the yield of the DMPO peroxy radical adduct is not affected by superoxide dismutase, it cannot be derived directly from superoxide. It is suggested instead that a sulfur peroxy radical intermediate may be involved,



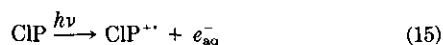
The yield of DMPO-OH follows the absorption curve of chlorpromazine fairly closely over the range 250 nm to 350 nm suggesting that the peroxy radical is formed from the triplet state of chlorpromazine via excitation into either the S_1 or S_2 energy levels (9). When promazine was irradiated under the same conditions DMPO trapped a peroxy adduct at pH 4 and the DMPO-OH decay product at pH 6.5 (9). Since the signal intensity of the DMPO-OH adduct did decrease in the presence of superoxide dismutase, superoxide must be generated during the irradiation of promazine. Similar findings have been reported by Decuyper et al. (11).

Phenothiazine Cation Radicals

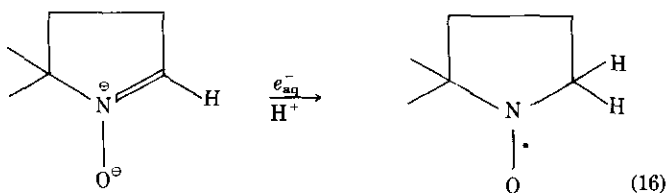
The free radical cations of phenothiazines may be generated by a variety of methods, including air oxidation in strong acid solutions, oxidation by horseradish peroxidase/H₂O₂ and electrolytic oxidation (12-14). Felmeister and Discher (15) have also detected the chlorpromazine cation radical (II) in aqueous acidic solutions of the drug irradiated with a mercury arc lamp.



Flash photolysis studies of either anaerobic or aerobic aqueous solutions of chlorpromazine at 347 nm (16) and 254 nm (17) have provided additional evidence for photoionization of the drug to yield the chlorpromazine cation radical (CIP^{•+}) and the aqueous electron (e_{aq}⁻)



More recently Motten et al. (9) have shown in a spin trapping study that when chlorpromazine is photolyzed at 270 nm (i.e., into the S₂ absorption band) a strong signal characteristic of the DMPO-H adduct is observed in addition to carbon- and oxygen-centered adducts. Since the DMPO-H signal was suppressed in the presence of N₂O, it was concluded that the spin trap had reacted with an electron [Eq. (16)].



However, in contrast to the flash photolysis study of Navaratnam and co-workers (16), no DMPO-H was observed when chlorpromazine was excited at 330 nm. The flash photolysis experiment used a 25 nsec flash, which is long compared to the chlorpromazine singlet state lifetime of only 1.3 nsec (R. D. Hall, unpublished data). Thus it is possible that a substantial steady state triplet population could have been produced during the flash photolysis experiment. Triplet-triplet absorption and subsequent electron ejection could under these conditions be pseudo first-order with respect to light intensity as was observed by Navaratnam et al. (16).

The cation radicals of chlorpromazine and other phenothiazines have been characterized by their absorption spectra (18-20) and electrochemical properties (21,22). The electron spin resonance (ESR) spectra of the phenothiazine cation radical in aqueous (23) and acetonitrile (24) solutions have been analyzed. However, it is only

recently that the aqueous solution spectra of cation radicals derived from chlorpromazine and the other phenothiazine tranquilizers have been successfully analyzed and simulated (12).

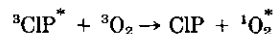
The ESR spectrum of the chlorpromazine cation radical generated by air oxidation in acid solution is shown in Figure 2. In addition, the aqueous ESR spectra of promazine, 2-chlorophenothiazine, promethazine, and trimeprazine have been recorded and analyzed (12).

Other Chlorpromazine Radicals

Forrest and co-workers (25) have reported the formation of a colorless free radical when dilute aqueous solutions of chlorpromazine were exposed to a sunlamp for 3 hr. The free radical character of the photoproduct was inferred from ESR spectra of a solid sample of the corresponding 2,4-dinitrophenylhydrazine derivative. However, Borg and Cotzias (13) failed to observe any free radicals in aqueous solutions of chlorpromazine irradiated with ultraviolet radiation. It was suggested that the ESR spectrum of the solid derivatives isolated by Forrest et al. was due to the formation of the cation radical during derivatization. Later, Piette and Forrest (26) reported the generation of a purple/blue substance by photooxidation of chlorpromazine. The ESR spectrum of this photoproduct indicated that it was not identical to the red chlorpromazine cation radical (II) formed under acidic conditions.

Singlet Oxygen

While singlet oxygen (¹O₂^{*}) is not a radical species it has been implicated in the phototoxicity of many chemicals (4). However, the generation of singlet oxygen during photoirradiation of the phenothiazines is still somewhat controversial. Davies and co-workers (10) have shown that, under aerobic conditions, there is no photodegradation of chlorpromazine dissolved in isopropanol. They have suggested that this is due to energy transfer from the triplet state of the drug to molecular oxygen to yield singlet oxygen:



Moore and Tamat (8) have found that the photode-

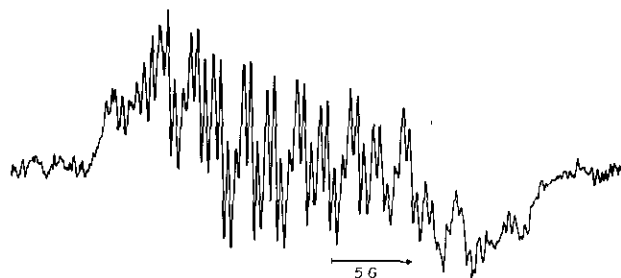


FIGURE 2. Electron spin resonance spectrum of the chlorpromazine cation radical in 20% DCl.

chlorination of chlorpromazine and prochlorperazine in methanol is also inhibited in the presence of oxygen.

Davies and co-workers have also reported (10) that, when chlorpromazine is irradiated in isopropanol in the presence of the singlet oxygen scavenger 2,5-dimethylfuran, there is rapid oxygen uptake which is inhibited by 1,4-diazabicyclo[2.2.2]octane (DABCO). Moore (27) has studied a series of phenothiazine tranquilizers in methanol solution and shown that 2,5-dimethylfuran stimulates oxygen uptake in the presence of promethazine, promazine, chlorpromazine, prochlorperazine, trifluoperazine and thioridazine. In contrast, Decuyper and co-workers (28) failed to detect singlet oxygen during the irradiation of ethanol solutions of promazine, trifluopromazine, methoxypropazine, and acepromazine using the method of Lion et al. (29). Similar results were obtained by the same workers in aqueous solution using cholesterol attached to polystyrene latex beads as the singlet oxygen monitor. More recently Hall and Chignell (unpublished results) have also failed to detect the 1270 nm emission from singlet oxygen during the photolysis of deuterium oxide solutions of chlorpromazine. However, a very weak emission was observed from chlorpromazine dissolved in oxygenated *n*-hexane. Thus it is not clear at the present time whether singlet oxygen does play a role in chlorpromazine phototoxicity.

Free Radical Mechanisms of Phenothiazine Photosensitivity

Phototoxicity

Upon irradiation the phenothiazines are known to elicit a wide variety of phototoxic responses (Table 2). While it seems likely that free radicals do play a significant role in phenothiazine phototoxicity, there is often only indirect evidence that these highly reactive chemical species are involved. For example, Decuyper and co-workers have shown that strand breakage in ϕ X174 DNA, caused by photoirradiation of promazine, methoxypropazine, or trifluopromazine, can be mimicked by the corresponding cation radicals generated either chemically or enzymatically (peroxidase/H₂O₂) (11). Merville and co-workers (41) have found the cation radicals of chlorpromazine, methoxypropazine, promethazine, trifluopromazine and acepromazine all cause a crosslinking of erythrocyte ghost membrane proteins which is similar to that observed when the membranes are photoirradiated in the presence of these same drugs. Other studies have shown that phenothiazine cation radicals are probably involved in the photoinduced inhibition of (Na⁺ + K⁺)-adenosinetriphosphatase by chlorpromazine, thioridazine, trifluopromazine, and trifluoperazine (46-48). It is of interest to note that the enzyme inhibition caused by irradiation in the presence of chlorpromazine could be reversed by cysteine or dithiothreitol (47). This suggests that the loss of enzyme

activity may be the result of oxidation of essential sulfhydryl groups by the cation radical. The cation radicals of phenothiazines are also known to react with ascorbic acid, NADH, various sulfhydryl compounds, α -tocopherol, adrenalin, and dihydroxyphenylalanine (49,51-53). However, the observation that photoionization of chlorpromazine occurs only upon excitation into the S₂ level ($\lambda_{\text{ex}} < 280$ nm) (9) makes it unlikely that direct photoformation of phenothiazine cation radicals by sunlight ($\lambda > 300$ nm) is important in cutaneous and ocular photosensitivity *in vivo*.

The highly reactive neutral radicals formed by the homolytic fission of the carbon-chlorine bond in chlorpromazine (Cl[•], P[•]) [Eq. (3)] and related phenothiazines may also play a role in phototoxicity. Decuyper and co-workers have shown that ϕ X174 DNA strand scission by photoirradiated chlorpromazine increases under anaerobic conditions (11). The denaturation of salmon sperm DNA by chlorpromazine and light is also enhanced in the absence of oxygen (44). Since oxygen would react rapidly with Cl[•] and P[•] it seems reasonable to assume that these radicals are involved. In this regard it is of interest to note that of the eight phenothiazines tested by Jose for photomutagenesis in *S. typhimurium*, only those that contained a chlorine atom (chlorpromazine, compazine, perphenazine) were active (31).

It is not clear at the present time what role active oxygen species (¹O₂, [•]OH, O₂^{•-}) play in phenothiazine phototoxicity. Kochevar and Lamola have found that oxygen caused only a small increase in chlorpromazine-induced photohemolysis of human erythrocytes (37). Similar results have been reported by Johnson (39). However, Copeland et al. (42) have observed that oxygen is necessary for the disruption of liposomes by light in the presence of chlorpromazine. In addition, the presence of oxygen enhances both the inactivation of ϕ X174 bacteriophage (32) and the strand breaking of ϕ X174 DNA (11).

The apparent inability of chlorpromazine to generate singlet oxygen upon irradiation (28) makes it unlikely that this active oxygen species is involved in the phototoxicity of this drug. Additional evidence for the lack of involvement of singlet oxygen has been provided by Nilson and co-workers, who have found that neither histidine nor β -carotene (quenchers of singlet oxygen) protects against chlorpromazine-induced photohemolysis of human erythrocytes (40). These workers also failed to demonstrate an effect of deuterium oxide (which increases the lifetime of ¹O₂^{*}) in the same system.

Decuyper and co-workers have suggested that superoxide may play a role in the strand breakage of ϕ X174 DNA observed during irradiation under aerobic conditions in the presence of promazine, trifluopromazine, and methoxypropazine (11). Recent spin-trapping studies by Motten et al. (9) have confirmed that superoxide is indeed generated during the irradiation of aqueous solutions of promazine. While the DMPO-hy-

droxyl radical adduct has been observed during the photoradiation of chlorpromazine, promazine, trifluorpromazine, and methoxypropazine (9,11) it seems unlikely that the hydroxyl radical is a primary photoproduct and therefore this reactive species cannot be involved in the phototoxicity of these drugs.

Kochevar and Lamola (37) have found that red cells are lysed in the dark by incubation with chlorpromazine solutions that had been previously irradiated in the absence of oxygen. This suggests that chlorpromazine photoproducts may be responsible for some of the photo-

toxic effects of this phenothiazine. Dimeric and higher polymeric photoproducts from chlorpromazine have been shown to cause red blood cell lysis in the absence of light (38).

Photoallergy

The photoallergic effect of chlorpromazine must be due to the covalent modification of proteins or other molecules to produce an antigen. The mechanism of the subsequent immunological response is presumably sim-

Table 2. Phototoxicity of phenothiazines.

| Phenothiazine | Biological systems | Phototoxic effect | Effect of oxygen ^a | Inhibitors | Reactive species identified | Reference |
|---|--|-----------------------------------|-------------------------------|--------------------------------------|---|-----------|
| Chlorpromazine | <i>S. typhimurium</i> | Mutagenesis | ND ^b | ND | ND | (30) |
| Chlorpromazine | Chinese hamster cells | Cell death | ND | ND | ND | (30) |
| Chlorpromazine, compazine, perphenazine | <i>S. typhimurium</i> | Mutagenesis | ND | ND | ND | (31) |
| Chlorpromazine, trifluorpromazine, methoxypropazine | φX174 bacteriophage | Inactivation | + | ND | Not ¹ O ₂ * | (32) |
| Chlorpromazine | <i>E. coli</i> , DNA, BSA ^c | Covalent binding | ND | ND | ND | (33) |
| Chlorpromazine | Human fibroblasts | Growth inhibition, DNA binding | ND | ND | ND | (34) |
| Chlorpromazine | Adenovirus 5 | DNA damage (single strand breaks) | ND | ND | ND | (35) |
| Chlorpromazine | <i>E. coli</i> | Cell death | ND | ND | ND | (36) |
| Chlorpromazine | Human erythrocytes | Hemolysis | + | ND | ND | (37) |
| Chlorpromazine | Human erythrocytes | Hemolysis | - | ND | ND | (38) |
| Chlorpromazine | Human erythrocytes | Hemolysis | - | ND | ND | (39) |
| Chlorpromazine | Human erythrocytes | Hemolysis | + | ND | ND | (40) |
| Methoxypropazine, promethazine, trifluorpromazine, acepromazine | Human erythrocyte membranes | Crosslinking | - | NaN ₃ | Cation radical OH [•] (methoxypropazine) | (41) |
| Chlorpromazine | Human erythrocyte membranes | Crosslinking | - | ND | P [•] | (41) |
| Chlorpromazine | Liposomes | Lysis | + | Cysteamine, tocopherol | ND | (42) |
| Chlorpromazine | RNA, DNA, Purines, pyrimidines | Covalent binding | ND | ND | ND | (43) |
| Chlorpromazine | φX174 DNA | Strand breakage | + - | ND | P [•] | (11) |
| Promazine, trifluorpromazine, methoxypropazine | φX174 DNA | Strand breakage | ++ | <i>tert</i> -BuOH, benzoate, formate | Cation radical OH [•] , O ₂ ^{-•} | (11) |
| Chlorpromazine | DNA (Salmon sperm) | Denaturation | - | ND | ND | (44) |
| Chlorpromazine | DNA | Intercalation | ND | ND | Cation radical | (45) |
| Chlorpromazine | (Na ⁺ + K ⁺)-ATPase | Inhibition | ND | ND | Cation radical | (46,47) |
| Thioridazine, trifluorpromazine, trifluoperazine | (Na ⁺ + K ⁺)-ATPase | Inhibition | ND | ND | Cation radical | (48) |
| Chlorpromazine | Ascorbate | Oxidation | ND | ND | Cation radical | (49) |
| Chlorpromazine | GSH, BSA | Oxidation of SH groups | + | ND | Cation radical | (50) |

^a Effects: - = inhibition of phototoxicity + = enhancement of phototoxicity.

^b ND = not determined.

^c BSA = bovine serum albumin.

ilar to other types of delayed hypersensitivity (54). Covalent binding of chlorpromazine to a variety of macromolecules (RNA, DNA, serum albumin, purines, pyrimidines) under the influence of light has been demonstrated (33,43). Davies and co-workers (10) have suggested that for chlorpromazine the promazinyl radical (P^{\cdot}) may be the reactive species that generates the antigen *in vivo*. However, the possibility that chlorpromazine photoproducts chemically modify biological macromolecules without covalently binding must also be considered.

Conclusion

Even though phenothiazines have been studied for decades, the detailed photochemistry of these substances is as yet incompletely known. Future research on this class of drugs should determine the role of active oxygen species $^1O_2^*$, $O_2^{\cdot-}$, and $\cdot OH$ in photosensitization; the detailed mechanism of the reaction of phenothiazines with oxygen; the chemistry of the promazinyl radical, P^{\cdot} ; the structure, characteristics, and possible role *in vivo* of the Forrest chlorpromazine radical (25); and the roles of stable photoproducts such as dimers and multimers, and sulfoxides in phototoxicity.

REFERENCES

- Pathak, M. A. Basic aspects of cutaneous photosensitization. In: *The Biologic Effects of Ultraviolet Radiation*, F. Urbach (Ed.), Pergamon Press, Oxford, 1969, pp. 480-511.
- Emmett, E. A. Phototoxicity from exogenous agents. *Photochem. Photobiol.* 30: 429-436 (1979).
- Kochevar, I. E. Photoallergic responses to chemicals. *Photochem. Photobiol.* 30: 437-442 (1973).
- Kochevar, I. E. Phototoxicity mechanisms: Chlorpromazine photosensitized damage to DNA and cell membranes. *J. Invest. Dermatol.* 76: 59-64 (1981).
- Zelickson, A. S., and Zeller, H. C. New and unusual reaction to chlorpromazine. *J. Am. Med. Assoc.* 188: 394-396 (1964).
- Epstein, J. H., Brunsting, L. A., Peterson, M. C., and Schwartz, B. E. Study of photosensitivity occurring during chlorpromazine therapy. *J. Invest. Dermatol.* 28: 329-338 (1957).
- Grant, F. W., and Greene, J. Phototoxicity and photonucleophilic aromatic substitution in chlorpromazine. *Toxicol. Appl. Pharmacol.* 23: 71-74 (1972).
- Moore, D. E., and Tamat, S. R. Photosensitization by drugs: Photolysis of some chlorine-containing drugs. *J. Pharm. Pharmacol.* 32: 172-177 (1980).
- Motten, A. G., Buettner, G. R., and Chignell, C. F.: Spectroscopic studies of cutaneous photosensitizing agents, VIII. A spin trapping study of light induced free radicals from chlorpromazine and promazine. *Photochem Photobiol.* 42: 9-16 (1983).
- Davies, A. K., Navaratnam, S., and Phillips, G. O. Photochemistry of chlorpromazine [2-chloro-N-(3-dimethylaminopropyl)phenothiazine] in propan-2-ol solution. *J. Chem. Soc. Perkin II* 1976: 25-29.
- Decuyper, J., Piette, J., Lopez, M., Merville, M.-P., and van de Vorst, A. Induction of deoxyribonucleic acid breakage by photoexcited promazine derivatives. *Biochem. Pharmacol.* 33: 4025-4031 (1985).
- Motten, A. G., and Chignell, C. F. ESR of cation radicals of phenothiazine derivatives. *Org. Mag. Res.* In press.
- Borg, D. C., and Cotzias, G. C. Interaction of trace metals with phenothiazine drug derivatives II Formation of free radicals. *Proc. Natl. Acad. Sci. (U.S.)* 48: 623-641 (1979).
- Piette, L. H., Bulow, G., and Yamazaki, I. Electron paramagnetic studies of the chlorpromazine free radical formed during enzymic oxidation by peroxidase-hydrogen peroxide. *Biochim. Biophys. Acta* 88: 120-129 (1964).
- Felmeister, A., and Discher, C. A. Photodegradation of chlorpromazine hydrochloride. *J. Pharm. Sci.* 53: 756-762 (1964).
- Navaratnam, S., Parsons, B. J., Phillips, G. D., and Davies, A. K. Laser flash photolysis study of the photoionization of chlorpromazine and promazine in solution. *J. Chem. Soc. Faraday Trans. I* 74: 1811-1819 (1978).
- Iwaoka, T., and Kondo, M. Mechanistic studies on the photooxidation of chlorpromazine in water and ethanol. *Bull. Chem. Soc. Japan* 47: 980-986 (1974).
- Thiery, C., Capette, J., Meunier, J., and Letterier, F. Étude spectroscopique d'une série de dérivés de la phenothiazine. *J. Chim. Phys.* 66: 134-139 (1968).
- Levy, L., Tozer, T. N., Tuck, L. D., and Loveland, D. B. Stability of some phenothiazine free radicals. *J. Med. Chem.* 15: 898-905 (1972).
- Cheng, H. Y., Sackett, P. H., and McCreery, R. L. Kinetics of chlorpromazine cation radical decomposition in aqueous buffers. *J. Am. Chem. Soc.* 100: 962-967 (1978).
- Sackett, P. H., and McCreery, R. L. Effect of structure on phenothiazine cation radical reactions in aqueous buffer. *J. Med. Chem.* 22: 1447-1453 (1979).
- Bodea, C., and Silberg, I. Recent advances in the chemistry of phenothiazines. *Adv. Heterocycl. Chem.* 9: 321-460 (1968).
- Lhoste, J. M., and Tonnard, F. Étude expérimentale et théorique de la résonance paramagnétique électronique des radicaux cations de la phénothiazine et de la phénoxazine. *J. Chim. Phys.* 63: 678-686 (1966).
- Gilbert, B. C., Hanson, P., Norman, R. O. C., and Sutcliffe, B. T. The cation-radical and the neutral radical from phenothiazine. *Chem. Commun.* 6: 161-164 (1966).
- Forrest, I. S., Forrest, F. M., and Berger, M. Free radicals as metabolites of drugs derived from phenothiazines. *Biochim. Biophys. Acta* 29: 441-442 (1958).
- Piette, L. H., and Forrest, I. S. Electron paramagnetic resonance studies of free radicals in the oxidation of drugs derived from phenothiazine *in vitro*. *Biochim. Biophys. Acta* 57: 419-420 (1962).
- Moore, D. E. Photosensitization by drugs. *J. Pharm. Sci.* 66: 1282-1284 (1977).
- Decuyper, J., Piette, J., Merville, M. P., Calberg-Bacq, C. M., and Van de Vorst, A. Lack of singlet oxygen formation by photoexcited promazine derivatives in aqueous and ethanolic solutions. *Radiat. Environ. Biophys.* 22: 231-234 (1983).
- Lion, Y., Delmelle, M., and Van de Vorst A. New method of detecting singlet oxygen production. *Nature* 263: 442-443 (1976).
- Ben-Hur, E., Prager, A., Green, M., and Rosenthal, I. pH-dependence of the phototoxic and photomutagenic effects of chlorpromazine. *Chem. Biol. Interact.* 29: 223-233 (1980).
- Jose, J. G. Photomutagenesis by chlorinated phenothiazine tranquilizers. *Proc. Natl. Acad. Sci. (U.S.)* 76: 469-472 (1979).
- Merville, M. P., Calberg-Bacq, C. M., Piette, J., Decuyper, J., and Van de Vorst, A. Phototoxicity of phenothiazine derivatives I. Inactivation and mutagenic effects on bacteriophage $\phi X174$. *Chem.-Biol. Interact.* 44: 261-274 (1983).
- Rosenthal, I., Ben-Hur, E., Prager, A., and Riklis, E. Photochemical reactions of chlorpromazine, chemical and biochemical implications. *Photochem. Photobiol.* 28: 591-594 (1978).
- Ljunggren, B., Cohen, S. R., Carter, D. M., and Wayne, S. I. Chlorpromazine phototoxicity, growth inhibition and DNA interaction in normal human fibroblasts. *J. Invest. Dermatol.* 75: 253-256 (1980).
- Day, R. S., and Dimattina, M. Photodynamic action of chlorpromazine on adenovirus 5: repairable damage and single strand breaks. *Chem.-Biol. Interact.* 17: 89-97 (1977).
- Matsuo, I., Ohkido, M., Fujita, H., and Suzuki, K. Chlorpromazine photosensitization. II. Failure to detect any involvement of DNA damage in the photodynamic killing of *Escherichia coli* in

- the presence of chlorpromazine. *Photochem. Photobiol.* 31: 175-178 (1980).
37. Kochevar, I. and Lamola, A. A. Chlorpromazine and protriptyline phototoxicity. Photosensitized, oxygen-independent red cell hemolysis. *Photochem. Photobiol.* 29: 791-796 (1979).
 38. Kochevar, I., and Hom, J. Photoproducts of chlorpromazine which cause red blood cell lysis. *Photochem. Photobiol.* 37: 163-168 (1983).
 39. Johnson, B. Cellular mechanisms of chlorpromazine photosensitivity. *Proc. Roy. Soc. Med. (London)* 67: 871-873 (1974).
 40. Nilsson, R., Swanbeck, G., and Wennersten, G. Primary mechanisms of erythrocyte photolysis induced by biological sensitizers and phototoxic drugs. *Photochem. Photobiol.* 22: 183-186 (1975).
 41. Merville, M. P., Piette, J., Decuyper, J., Calberg-Bacq, C. M., and Van de Vorst, A. Phototoxicity of phenothiazine derivatives II Photosensitized crosslinking of erythrocyte membrane proteins. *Chem.-Biol. Interact.* 44: 275-287 (1983).
 42. Copeland, E. S., Alving, C. R., and Grenan, M. M. Light induced leakage of spin label marker from liposomes in the presence of phototoxic phenothiazines. *Photochem. Photobiol.* 24: 41-48 (1976).
 43. Kahn, G., and Davis, B. P.: *In vitro* studies on longwave ultraviolet light dependent reactions of the skin photosensitizer chlorpromazine with nucleic acids, purines and pyrimidines. *J. Invest. Dermatol.* 55: 47-52 (1970).
 44. Schothorst, A. A., Suurmond, D., and Schouten, R. Photochemical damage to DNA treated with chlorpromazine and near UV radiation under aerobic and anerobic conditions. *Biochem. Pharmacol.* 38: 659-664 (1983).
 45. Ohnishi, S. and McConnell, H. M. Interaction of the radical ion chlorpromazine with deoxyribonucleic acid. *J. Am. Chem. Soc.* 87: 2293 (1965).
 46. Akera, T., and Brody, T. M. Inhibition of brain sodium- and potassium-stimulated adenosine triphosphatase activity by chlorpromazine free radical. *Mol. Pharmacol.* 4: 600-612 (1968).
 47. Akera, T., and Brody, T. M. The interaction between chlorpromazine free radical and microsomal sodium- and potassium-activated adenosine triphosphatase. *Mol. Pharmacol.* 5: 605-617 (1969).
 48. Gubitz, R. H., Akera, T., and Brody, T. M. Comparative effects of substituted phenothiazines and their free radicals on ($\text{Na}^+ + \text{K}^+$)-activated adenosine triphosphatase. *Biochem. Pharmacol.* 22: 1229-1235 (1973).
 49. Klein, N. A., and Toppen, D. L. Kinetics and mechanism of the reduction of chlorpromazine radical by ascorbic acid. *J. Am. Chem. Soc.* 100: 4541-4543 (1978).
 50. Hoffman, A. J. and Discher, C. A. Chlorpromazine sensitized photodynamic oxidation of sulfhydryl groups in biological molecules. *Arch. Biochem. Biophys.* 126: 728-730 (1968).
 51. Wollemann, M. *In vitro* oxidation of DPNH by free radicals of chlorpromazine. *Biochem. Pharmacol.* 12: 757-759 (1963).
 52. Felmeister, A., Schaubman, R., and Howe, H. Dismutation of a semiquinone free radical of chlorpromazine. *J. Pharm. Sci.* 54: 1589-1593 (1965).
 53. Mahmood, J. S., Packer, J. E., Searle, A. J. F., Willson, R. L., and Wolfenden, B. S. Chlorpromazine, vitamin C, vitamin E and catecholamines: Direct observations of free radical interactions. In: *Phenothiazines and Structurally Related Drugs: Basic and Clinical Studies*. Elsevier-North Holland, Amsterdam, 1980, pp. 103-106.
 54. Harber, L. C. and Baer, R. L. Classification and characteristics of photoallergy. In: *Biologic Effects of Ultraviolet Radiation* (F. Urbach, Ed.), Pergamon Press, Oxford 1969, pp. 519-527.