Toxicology and Applied Pharmacology 313 (2016) 131–137

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

# Enhanced photo(geno)toxicity of demethylated chlorpromazine metabolites

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ARTICLE INFO

*Article history:*
Received 5 August 2016
Accepted 25 October 2016
Available online 27 October 2016

*Keywords:*
Comet assay
Excited states
Laser flash photolysis
Metabolites
Photosensitized DNA damage
Phototoxicity

ABSTRACT

Chlorpromazine (CPZ) is an anti-psychotic drug widely used to treat disorders such as schizophrenia or manic-depression. Unfortunately, CPZ exhibits undesirable side effects such as phototoxic and photoallergic reactions in humans. In general, the influence of drug metabolism on this type of reactions has not been previously considered in photosafety testing. Thus, the present work aims to investigate the possible photo(geno)toxic potential of drug metabolites, using CPZ as an established reference compound. In this case, the metabolites selected for the study are demethylchlorpromazine (DMCPZ), didemethylchlorpromazine (DDMCPZ) and chlorpromazine sulfoxide (CPZSO). The demethylated CPZ metabolites DMCPZ and DDMCPZ maintain identical chromophore to the parent drug. In this work, it has been found that the nature of the aminoalkyl side chain modulates the hydrophobicity and the photochemical properties (for instance, the excited state lifetimes), but it does not change the photoreactivity pattern, which is characterized by reductive photodehalogenation, triggered by homolytic carbon-chlorine bond cleavage with formation of highly reactive aryl radical intermediates. Accordingly, these metabolites are phototoxic to cells, as revealed by the 3T3 NRU assay; their photo-irritation factors are even higher than that of CPZ. The same trend is observed in photogenotoxicity studies, both with isolated and with cellular DNA, where DMCPZ and DDMCPZ are more active than CPZ itself. In summary, side-chain demethylation of CPZ, as a consequence of Phase I biotransformation, does not result a photodetoxification. Instead, it leads to metabolites that exhibit in an even enhanced photo(geno)toxicity.



## 1. Introduction

Chlorpromazine (CPZ) is an anti-psychotic agent that belongs to the family of phenothiazines. From a clinical standpoint, it is widely used to treat psychotic disorders such as schizophrenia or manic-depression. Unfortunately, CPZ has often been reported as photosensitizing agent, with undesirable side effects such as phototoxic and photoallergic reactions in humans (Epstein et al., 1957; Fitzpatrick et al., 1963; Satanove and McIntosh, 1967; Johnson, 1974; Ljunggren and Moller, 1977; Kochevar et al., 1984; Motten et al., 1985; Beijersbergen van Henegouwen, 1997). In addition, CPZ photoproducts like promazine (PMZ) and chlorpromazine sulfoxide (CPZSO) have revealed toxic effects on primary cultures of hepatocytes (Castell et al., 1987). More recently, toxic epidermal necrolysis induced by CPZ upon sunlight exposure has been noticed (Huang et al., 2010). The phototoxic activity of drugs can be related with their genotoxic and mutagenic potential; in this context, CPZ is able to promote DNA photodamage (De Mol and Busker, 1984; Kochevar et al., 1984; Viola et al., 2003).

From the urine of psychiatric patients, CPZ metabolites have been identified (Beckett et al., 1963) revealing that the metabolic pathways during Phase I lead to demethylation, sulphoxidation and hydroxylation (Hartmann et al., 1983; Chetty et al., 1994). Thus, biotransformation of CPZ results in a variety of derivatives, which have been considered for establishing the pharmacological and toxicological profile (Wójcikowski et al., 2010).

As a general rule, metabolism converts hydrophobic chemicals into more hydrophilic derivatives that can be easily eliminated through the urine. However, in certain cases, drug-metabolizing enzymes can also produce electrophilic metabolites that react with cellular macromolecules such as DNA, RNA, and proteins, causing cell death and organ toxicity (Pearson and Wienkers, 2009).

*Abbreviations:* CPZ, chlorpromazine; PMZ, promazine; DMCPZ, demethylchlorpromazine; DDMCPZ, didemethylchlorpromazine; CPZSO, chlorpromazine sulfoxide; EPR, electron paramagnetic resonance; Fpg, *E. coli* formamidopyrimidine DNA glycosylase; Endo III, *E. coli* endonuclease III; Endo V, T4 endonuclease V; FSK, fibroblasts; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; TAE, tris-acetate-EDTA; MNP, 2-methyl-2-nitrosopropane; NRU, neutral red uptake; PIF, photo-irritation-factor.

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http://dx.doi.org/10.1016/j.taap.2016.10.024
0041-008X/

Assessment of the phototoxic potential of sunlight-absorbing drugs is highly recommended by health authorities (FDA, EMEA) (EMEA, 2002; FDA, 2003) but the influence of metabolism is not considered in the currently used assays. For that reason, identification of reactive drug metabolites with phototoxic or adduct forming capability is a major challenge. Hence, the goal of the present work is to investigate the possible photo(geno)toxic potential of drug metabolites, using CPZ as an established reference compound. In this case, the metabolites selected for the study (see Fig. 1) are demethylchlorpromazine (DMCPZ), didemethylchlorpromazine (DDMCPZ) and chlorpromazine sulfoxide (CPZSO). Both DMCPZ and DDMCPZ maintain the CPZ chromophore unaltered, so modulation of the photobiological effects would be associated with the nature of the aminoalkyl side-chain. By contrast, CPZSO displays a modified tricyclic aromatic core, which could be responsible for major changes in the phototoxicological properties.

The proposed multidisciplinary approach encompasses from photochemical (steady-state and laser flash photolysis) to spectroscopic (Electron Paramagnetic Resonance, EPR) and biological studies (neutral red uptake viability test, gel electrophoresis, comet assay) in order to obtain mechanistic insight into the involved process.

## 2. Materials and methods

### 2.1. General

All solvents (HPLC grade) and chemicals were commercially available and used without additional purification. Neutral red-based *in vitro* toxicology assay kit, and DNA repair enzymes *E. coli* formamidopyrimidine DNA glycosylase (Fpg), *E. coli* endonuclease III (Endo III) were purchased from Sigma Aldrich (Madrid, Spain). Supercoiled circular pBR322 DNA, SYBR Safe DNA gel stain and DNA repair enzyme T4 endonuclease V (Endo V) were provided by Roche Diagnostics (Barcelona, Spain), Invitrogen (Madrid, Spain) and Ecogen (Barcelona, Spain), respectively. Phosphate buffered saline solution (PBS, pH 7.4, 0.01 M) was prepared by dissolving Sigma tablets in the appropriate amount of deionized water. Reagent kit for single cell electrophoresis assay was supplied by Trevigen (Barcelona, Spain).

### 2.2. Synthesis of metabolites

Synthesis of DMCPZ was achieved in two-steps following the described demethylation procedure (Kitamura et al., 2000). In the first step, α-chloroethyl chloroformate (ACE-Cl) was added to an ethylene dichloride solution of CPZ in order to form the intermediate ACE-CPZ, whose methanolysis afforded DMCPZ hydrochloride. As regards, DDMCPZ hydrochloride it was obtained by reduction of the 7-chlorophenothiazinyl nitrile using $LiAlH_4$. Soxhlet extraction was performed in diethyl ether for 3 days, at 40 °C as in the original procedure described for a related compound (Zhou et al., 2010). Finally, CPZSO was prepared from CPZ by oxidation in aqueous nitrous acid, at room temperature, following the reported method (Owens et al., 1989).

All reactions were monitored by analytical TLC with silica gel 60 F254 and revealed with ammonium molybdate reagent. The crudes were purified through silica gel 60 (0.063–2 mm). $^1$H NMR and $^{13}$C NMR spectra were recorded in $CDCl_3$ or $CD_3OD$ as solvents on a Bruker AC-300 at 300 and 75 MHz respectively; NMR chemical shifts are reported in ppm downfield from an internal solvent peak.

**DMCPZ.** $^1$H-NMR ($CD_3OD$, 300 MHz) δ 2.19 (m, 2H), 2.64 (s, 3H), 3.10 (m, 2H), 4.06 (t, $J$ = 6.3 Hz, 2H), 6.94–7.28 (m, 7H). $^{13}$C NMR ($CD_3OD$, 75 MHz) δ 24.7, 33.7, 45.2, 48.2, 117.4, 117.6, 123.9, 124.6, 126.0, 127.0, 128.7, 129.0, 129.3, 134.7, 145.6, 147.9.

**DDMCPZ.** $^1$H-NMR ($CD_3OD$, 300 MHz) δ 2.13 (m, 2H), 2.98–3.09 (m, 2H), 4.08 (t, $J$ = 6.4 Hz, 2H), 6.95–7.25 (m, 7H). $^{13}$C NMR ($CD_3OD$, 75 MHz) δ 26.0, 38.6, 45.2, 117.4, 117.6, 123.9, 124.6, 126.1, 127.0, 128.6, 128.9, 129.3, 134.7, 145.7, 148.0.

**CPZSO.** $^1$H-NMR ($CDCl_3$, 300 MHz,) δ 2.17 (m, 2H), 2.37 (s, 6H) 2.59 (m, 2H), 4.37 (m, 2H), 7.22–7.25 (dd, $J$ = 8.4, 1.8 Hz, 1H), 7.30–7.32 (dd, $J$ = 7.8, 1.2 Hz, 1H), 7.53–7.55 (d, $J$ = 8.1 Hz, 1H), 7.60–7.69 (m, 2H), 7.85–7.88 (d, $J$ = 8.4 Hz, 1H), 7.93–7.95 (dd, $J$ = 7.8, 1.5 Hz, 1H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 24.3, 45.1, 45.7, 56.1, 116.3, 116.4, 122.2, 122.5, 131.4, 132.6, 133.1, 138.0, 139.1, 139.6.

### 2.3. Irradiation equipment

For all *in vitro* photosensitization assays, a photoreactor model LZC-4 (Luzchem, Canada) equipped with 14 lamps for top and side irradiation ($\lambda_{max}$ = 350 nm, Gaussian distribution) was used as the UVA light source. All irradiations were performed through the lid of the plates and the temperature was controlled by ventilation during the irradiation step.

### 2.4. In Vitro 3T3 neutral red uptake (NRU) phototoxicity test

BALB/c 3T3 fibroblasts cell line was grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum

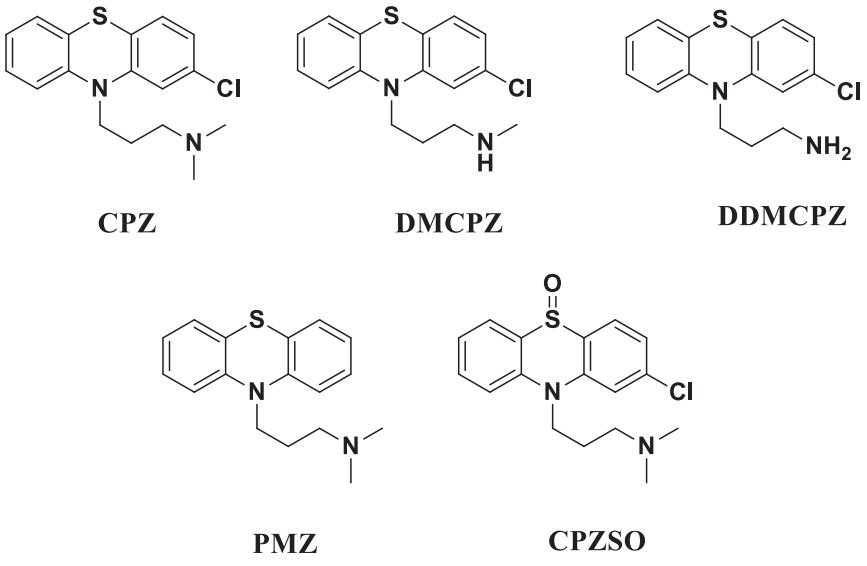


**Fig. 1.** Chemical structures of CPZ, DMCPZ, DDMCPZ, PMZ and CPZSO.