Toxicology 330 (2015) 44–54

Contents lists available at ScienceDirect

Toxicology

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

Cytotoxicity of phenothiazine derivatives associated with mitochondrial dysfunction: A structure-activity investigation

Priscila A. de Faria [a], Fernanda Bettanin [a], Rodrigo L.O.R. Cunha [a], Edgar J. Paredes-Gamero [b], Paula Homem-de-Mello [a], Iseli L. Nantes [a], Tiago Rodrigues [a,*]

[a] Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, SP, Brazil
[b] Departamento de Bioquímica, Universidade Federal de São Paulo, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 12 February 2015
Accepted 12 February 2015
Available online 14 February 2015

Keywords:
HTC cells
Cytotoxicity
Mitochondrial permeability transition
Phenothiazine
Cell death
Structure-activity

ABSTRACT

Phenothiazine derivatives are neuroleptic drugs used in the treatment of schizophrenia and anxiety. Several side effects are described for these drugs, including hepatotoxicity, which may be related to their cytotoxic activity. Working with isolated rat liver mitochondria, we previously showed that phenothiazine derivatives induced the mitochondrial permeability transition associated with cytochrome *c* release. Since the mitochondrial permeabilization process plays a central role in cell death, the aim of this work was to evaluate the effects of five phenothiazine derivatives (chlorpromazine, fluphenazine, thioridazine, trifluoperazine, and triflupromazine) on the viability of hepatoma tissue culture (HTC) cells to establish the structural requirements for cytotoxicity. All phenothiazine derivatives decreased the viability of the HTC cells in a concentration-dependent manner and exhibited different cytotoxic potencies. The EC_{50} values ranged from 45 to 125 μM, with the piperidinic derivative thioridazine displaying the most cytotoxicity, followed by the piperazinic and aliphatic derivatives. The addition of the phenothiazine derivatives to cell suspensions resulted in significant morphological changes and plasma membrane permeabilization. Octanol/water partition studies revealed that these drugs partitioned preferentially to the apolar phase, even at low pH values (≤4.5). Also, structural and electronic properties were calculated employing density functional theory. Interestingly, the phenothiazine derivatives promoted an immediate dissipation of the mitochondrial transmembrane potential in HTC cells, and the EC_{50} values were closely correlated with those obtained in cell viability assays, as well as the EC_{50} for swelling in isolated mitochondria. These results significantly contribute to improving our understanding of the specific structural requirements of the phenothiazine derivatives to induce cell death and suggest the involvement of the mitochondrial permeability transition in phenothiazine-induced cytotoxicity in HTC cells.

1. Introduction

Phenothiazine derivatives (PTZs) are drugs used clinically in the treatment of schizophrenia, psychosis, and anxiety (Lehmann and Ban, 1997). Some of the derivatives have also been used effectively as anti-emetic drugs (Allan, 1987; Bhargava and Chandra, 1963; McCabe and Maraveyas, 2003). Chemically, PTZs comprise a class of heterocyclic compounds that have a tricyclic phenothiazinic nucleus (PHT) related to anthracene, with the central ring containing sulfur and nitrogen atoms. PTZs are substituted at the 2- and 10-positions, with the nature of the ligand at position 10 modulating the pharmacological antipsychotic activity, while

Abbreviations: CMF-BSS, calcium- and magnesium-free buffered saline solution; CPZ, chlorpromazine; DMEM, Dulbecco's modified Eagle'medium; EC_{50}, half maximal effective concentration; FP, fluphenazine; HTC, hepatoma tissue culture; IMP, imipramine; LDH, lactate dehydrogenase; MPT, mitochondrial permeability transition; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; O/W, *n*-octanol/water; PHT, phenothiazinic nucleus; PI, propidium iodide; PTZ, phenothiazine derivative; TFP, trifluoperazine; TFPZ, triflupromazine; TR, thioridazine; $\Delta\Psi$, mitochondrial transmembrane potential; DFT, density functional theory.

* Corresponding author at: Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Rua Santa Adélia, 166, Bloco A, Torre 3, Sala 623, Santo André, SP CEP 09210-170, Brazil. Tel.: +55 11 4996 8371.

E-mail addresses: priscilaafonsofaria@yahoo.com.br (P.A. de Faria), fernandabettanin@gmail.com (F. Bettanin), rodrigo.cunha@ufabc.edu.br (R.L.O.R. Cunha), edgar.gamero@unifesp.br (E.J. Paredes-Gamero), paula.mello@ufabc.edu.br (P. Homem-de-Mello), ilnantes@ufabc.edu.br (I.L. Nantes), tiago.rodrigues@ufabc.edu.br (T. Rodrigues).

http://dx.doi.org/10.1016/j.tox.2015.02.004
0300-483X/

the presence of electron withdrawing groups at position 2 increases the therapeutic efficacy (Baldessarini and Tarazi, 2001; Biel et al., 1978). Based on the 10-position substituents, PTZs can be classified as aliphatic, piperazinic, or piperidinic derivatives (Chen et al., 2002; Fenton et al., 2007). Such a peculiar chemical structure confers an amphiphilic character on PTZs, which results in the well-described property of PTZ interactions with membranes modifying their biophysical properties and consequently modulating biological processes (Hendrich et al., 2001, 2007; Rodrigues, 2007; Wesołowska, 2011; Wesołowska et al., 2004).

Although extrapiramidal symptoms are the most common side effects described for patients on PTZs (Cole and Clyde, 1961), severe hepatotoxicity has also been associated with the clinical use of these drugs, including acute intrahepatic cholestasis (Andrade et al., 2007; Goodman, 2002; Moradpour et al., 1994), steatosis (Brind, 2007), and hepatitis (Weiden and Buckner, 1973). Chlorpromazine, an aliphatic phenothiazine derivative, exhibited higher cytotoxicity in liver cells than promazine, and this effect seems to be related to its capacity to interact with membranes (Salhab and Dujovne, 1986). The association of tricyclic antidepressants and phenothiazines was related to increased levels of aminotransferases as liver injury markers, and it was proposed that the tricyclic ring could be involved in the observed hepatotoxicity (Remy et al., 1995). Nevertheless, the structural requirements and the molecular mechanisms of PTZ-induced hepatotoxicity remain unclear. It was proposed that the cytochrome P450-meadiated bioactivation of these drugs could be responsible for the hepatotoxicity observed (Wen and Zhou, 2009). We have previously shown that high concentration of PTZs (>25 μM) induce the mitochondrial permeability transition (MPT) in rat liver mitochondria associated with the release of cytochrome *c* (Cruz et al., 2010), features that are intrinsically related to cell death. In fact, PTZs have been reported to induce cytotoxicity in several cell lines (Hieronymus et al., 2000; Karmakar et al., 2001; Shin et al., 2013; Zhelev et al., 2004). In this study, we examined the effects of five commercially available PTZs (Fig. 1; chlorpromazine, CPZ; fluphenazine, FP; thioridazine, TR; trifluoperazine, TFP; and triflupromazine, TFPZ) on the viability of hepatoma tissue culture (HTC) cells as a model for studying cytotoxicity and determining the structural features of the PTZs that are responsible for this effect. We also investigated the involvement of mitochondrial dysfunction in PTZ-induced cell death.

2. Materials and methods

2.1. Materials

Phenothiazine derivatives (CPZ, FP, TR, TFP, and TFPZ) and related drugs (IMP and PHT) were purchased from Sigma–Aldrich (St Louis, MO, USA). All other chemicals were acquired as commercial products at the highest purity grade available, and the solutions and buffers were prepared with double distilled-deionized water (mixed bed ion exchanger, Milli-Q system; Millipore Corp., Bedford, MA, USA). Also, the stock solutions of all drugs used here were prepared in Milli-Q water, except for PHT, which was solubilized in dimethylsulfoxide (DMSO).

2.2. Cell culture

The HTC cells (hepatoma tissue culture; Thompson et al., 1966) were cultivated in high-glucose Dulbecco's modified Eagle's medium (DMEM; Sigma Chemical Co.) supplemented with 10% fetal bovine serum (FBS) (heat inactivated, South American origin; GIBCO-Invitrogen Corp., Grand Island, NY, USA), 100 U/mL penicillin (GIBCO-Invitrogen Corp.), and 100 μg/mL streptomycin (GIBCO-Invitrogen Corp.) at 37 °C in a 5% CO_2 atmosphere (Sanyo MCO-20AIC incubator; Sanyo Electric Co., Ltd. Osaka, Japan). For the experiments, cells were washed twice with a calcium- and magnesium-free buffered saline solution (CMF-BSS), detached from the flasks with trypsin/EDTA (GIBCO-Invitrogen Corp.), and resuspended in the supplemented media.

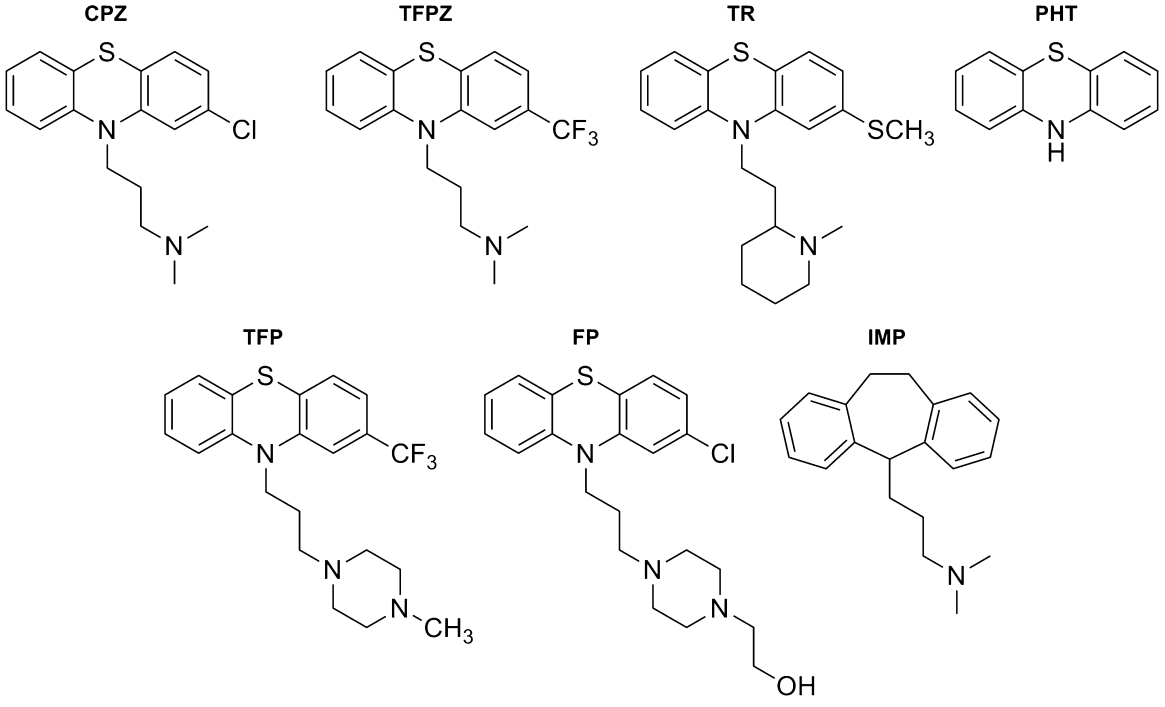

Fig. 1. Molecular structure of the phenothiazine derivatives, phenothiazinic nucleus, and imipramine. The imipramine was included to test the cytotoxic influence of the sulfur atom at the core of the phenothiazine derivatives. CPZ, chlorpromazine; TFPZ, triflupromazine; TR, thioridazine; PHT, phenothiazinic nucleus; TFP, trifluoperazine; FP, fluphenazine; IMP, imipramine.