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ORIGINAL ARTICLE

Short communication: Chlorpromazine causes a time-dependent decrease of lipids in *Saccharomyces cerevisiae*

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ABSTRACT

Chlorpromazine (CPZ) is still a commonly prescribed antipsychotic which causes poorly understood idiosyncratic toxicity such as cholestasis, phospholipidosis and steatosis. CPZ has diverse cellular targets and exerts various toxicity mechanisms whose exploration is necessary to understand CPZ side effects. We report here that CPZ causes a decrease of total lipid content in *Saccharomyces cerevisiae* at the same dose range as that used on mammalian cells. The observed lipid decrease was obvious after 4 and 9 hours of treatment, and disappeared after 24 hours due to cells adaptation to the chemical stress. The inhibitory effect of CPZ was antagonized by the antioxidant N-acetyl L-cysteine and is likely caused by the parent compound. The obtained results demonstrate that yeast model is valid to investigate the involved CPZ toxicity mechanisms, particularly in terms of lipids alteration. This would contribute to understand CPZ side effects in simple model and reduce experimentation on animals.

KEY WORDS: phenothiazine; chlorpromazine toxicity; *Saccharomyces cerevisiae*; lipids; oxidative stress

Introduction

Chlorpromazine (CPZ) is a first generation antipsychotic phenothiazine which is still commonly prescribed (Dudley *et al.,* 2017). CPZ mediates its antipsychotic effects by blocking the dopamine D2 receptors (Suzuki *et al.,* 2013). The therapeutic use of CPZ is associated with poorly understood idiosyncratic adverse reactions, mainly hepatotoxicity, due to its numerous molecular targets. CPZ toxicity mechanisms are diverse with an emphasis on oxidative stress and alteration of the expression of genes involved in transport across the cell membrane and in lipid metabolism (Antherieu *et al.,* 2013; Dejanović *et al.,* 2017; Hu & Kulkarni, 2000). CPZ causes also membrane structure alteration (Kamada *et al.,* 1995; Morgan *et al.,* 2019).

In different experimental models, CPZ induces phospholipidosis and steatosis (Bachour-El Azzi *et al.,* 2014). Phospholipidosis is characterized by intra-cellular accumulation of phospholipids as lamellar bodies (Anderson & Chan, 2016). Moreover, phenothiazines, including CPZ, cause alteration of the lipid profile by affecting the activity of several enzymes involved in lipid metabolism (Hoshi & Fujino, 1992; Ide & Nakazawa, 1980; Martin *et al.,* 1986). In humans, treatment with phenothiazines elevates serum triglyceride and total cholesterol levels (Saari *et al.,* 2004). These changes in lipid metabolism could also be mediated by the effect of CPZ on microtubules and possibly the traffic of vesicles inside the cell (Thyberg *et al.,* 1977), in addition to the alteration of lipid metabolism-related enzymes.

Investigating the mechanisms of CPZ toxicity, such as cholestasis and lipids alteration, in human is difficult for ethical reasons. Although diverse *in vitro* models are in use (Morgan *et al.,* 2019), CPZ toxic effects remain ambiguous. CPZ toxicity may be due to the parent molecule as well as to its metabolites (Abernathy *et al.,* 1977; Parmentier *et al.,* 2013; Tavoloni & Boyer, 1980; Yeung *et al.,* 1993) such as (mono-N-demethylated CPZ, di-N-demethylated CPZ, CPZ sulfoxide). These metabolites are not detected in *Saccharomyces cerevisiae* (our unpublished data) probably due to the lack of cytochromes P450 homologous to CYP1A2 and CYP3A4 which catalyze CPZ

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biotransformation (De Filippi *et al.,* 2007; Wójcikowski *et al.,* 2010).

In this study on *Saccharomyces cerevisiae*, we investigated CPZ toxic effects on yeast lipid content and we indirectly assessed the involvement of oxidative stress. CPZ acted in a similar manner on *Saccharomyces cerevisiae* relative to mammalian cells in terms of oxidative stress involvement. Total lipid amount was lower in CPZ-treated cells. Our data validate the use of the yeast model to investigate CPZ toxicity mechanisms, especially regarding lipid alteration.

Materials and methods

Saccharomyces cerevisiae BY4741 wild type strain was supplied by Euroscarf-Germany and used in our experiments. Yeast cells were grown at 30°C in Yeast Peptone Dextrose (YPD, Sigma-Germany) rich medium (1% yeast extract, 2% peptone and 2% dextrose), pH=7.4. Yeast cells from the mid exponential growth phase were treated with CPZ (Abcam Biochemicals, USA) and growth was assessed by spectrophotometry (630 nm) in a dose- and time-dependent manner. N-acetyl L-cysteine (NAC) was from Armesco USA.

Yeast cells were lysed mechanically and total proteins were measured using the Bradford assay. Total lipid extraction was performed using ice-cold chloroform/methanol (2:1) as previously described (Knittelfelder & Kohlwein, 2017). Total lipid measurement was performed by gravimetry using a precision balance (Boeco, Germany, d=0.1 mg) as well as by spectrophotometry at 580 nm using Sudan black as previously described (Thakur *et al.,* 1989).

Table 1. Effect of CPZ ± NAC on lipid content in *S. cerevisiae*.

Lipid (relative to control)	CPZ 20 µM	CPZ 50 µM	NAC 5 mM	NAC+CPZ 50 µM
4 hours	0.30±0.23*	0.37±0.15*	nd	nd
9 hours	nd	0.53±0.08*	0.89±0.07	1.07±0.15#
24 hours	nd	0.85±.14	1.11±0.04	1.62±0.5

Yeast cells from the mid exponential growth phase were treated with the indicated doses and total lipids extracted and assessed by spectrophotometry and gravimetry at the indicated time points. The results were normalized to OD values of the culture at each time point, and then expressed relative to the value in the control untreated cells. The values correspond to the average±SEM of 4 to 9 independent experiments. Statistically significant difference compared to the control (*) and to CPZ 50 µM (#), $p<0.05$. nd, not determined.

Table 2. Effect of CPZ ± NAC on total protein content in *S. cerevisiae*.

	Control	CPZ 50 µM	NAC 5mM	NAC+CPZ 50µM
Proteins (mg/ml)	5.73±0.85	2.69±0.65*	5.68±0.37	4.42±1.72#

Yeast cells from the mid exponential phase were treated with the indicated dose for 24 hours and then collected and lysed. Total proteins were measured by spectrophotometry. The results are normalized to OD values of the culture. The values correspond to the average±SEM of 4 to 9 independent experiments. Statistically significant difference compared to the control (*) and to CPZ 50 µM (#), $p<0.05$.

Data were expressed as means ± SEM from four to nine independent experiments. They were statistically analyzed using GraphPad Prism software through analysis of variance (ANOVA) followed by Dunnett's *post-hoc* test, or F-test. The criterion of significance for statistical tests was $p<0.05$. The half maximal inhibitory concentration IC50 of CPZ was calculated from nonlinear regression of dose-response data (doses used up to 200 µM) based on the four parameter logistic function, using GraphPad Prism software (GraphPad Software, La Jolla, CA).

Results and discussion

CPZ inhibited yeast growth in a dose and time-dependent manner. CPZ IC_{50} was 28.08±1.03 µM and 43.83±1.02 µM after 6 and 24 hours of treatment, respectively. This result is not graphically illustrated, but in agreement with our previous work (Sayyed *et al.,* 2019). CPZ toxic effect was antagonized by the antioxidant NAC, thereby demonstrating oxidative stress as a main toxicity mechanism. Yeast cells are less sensitive to CPZ after 24 hours due to their ability to develop a chemical stress resistance response, which is in agreement with previous reports (Al-Attrache *et al.,* 2018; De Filippi *et al.,* 2007; Simpson & Ashe, 2012). Since CPZ has many molecular targets *in vivo* and *in vitro* (De Filippi *et al.,* 2007; Ros *et al.,* 1979; Thyberg *et al.,* 1977), its toxic effects deserve investigation in order to help understanding its toxicity *in vivo*. Validity of *Saccharomyces cerevisiae* as a model to investigate CPZ toxicity is valuable since it helps reducing experimentation on animals.

Our results show that CPZ at 20 and 50 µM decreases total lipids up to 70% after 4 hours of treatment. Lower, but significant, decrease in percentage was obtained after 9 hours (46%) while the decrease was not significant after 24 hours (Table 1). In the presence of NAC, the effect of CPZ on lipid content disappeared suggesting the involvement of oxidative stress in the CPZ-caused lipid decrease. The lack of CPZ effect after 24 hours is probably attributed, at least partially, to an adaptation mechanism of yeast to the chemical stress. The effects of CPZ on lipid content of *S. cerevisiae* could be due to alteration of the activity of enzymes involved in lipid metabolism. In fact, in animal cells, it was reported that CPZ alters the expression of many lipid metabolism-related genes, including ADRP (Adipose Differentiation-Related Protein) and Perilipin-4 genes which are involved in the formation of lamellar vesicles and Acyl-CoA (Bachour-El Azzi *et al.,* 2014; Hoshi & Fujino, 1992; Jassim *et al.,* 2012; Martin *et al.,* 1986). In addition, CPZ inhibits triacylglycerol synthesis by inhibiting the enzyme phosphatidate phosphohydrolase and thus the inhibition of diacylglycerol formation in rats (Bowley *et al.,* 1977; Ide & Nakazawa, 1980, 1981). CPZ effects on yeast are likely due to the parent drug since this yeast does not have enzymes equivalent to those involved in chlorpromazine metabolism in human (e.g. CYP1A2, CYP3A4,…) (De Filippi *et al.,* 2007; Wójcikowski *et al.,* 2010). None of the CPZ metabolites

found in human (mono-N-demethylated CPZ, di-N-demethylated CPZ, CPZ sulfoxide, 7-OH CPZ) were detected in *Saccharomyces cerevisiae* (our unpublished data). Therefore, this yeast is a simple and valid model to investigate effects of CPZ itself on lipid alteration. In mammalian cell models, metabolites of CPZ contribute to its toxicity (Abernathy *et al.,* 1977; Parmentier *et al.,* 2013; Yeung *et al.,* 1993).

Our results also show that 50 µM of CPZ significantly decreases total protein content of *S. cerevisiae* by 52%, and that the co-treatment with NAC reverses this inhibition after 24 hours (Table 2). These results demonstrate that protein synthesis and/or turn over are targets of CPZ and that oxidative stress is involved in CPZ toxicity in *S. cerevisiae.* This result is in agreement with previous studies on *S. cerevisiae* and NIH-3T3 cells, suggesting involvement of similar mechanisms of CPZ toxicity in yeast and mammalian cells (De Filippi *et al.,* 2007; Deloche *et al.,* 2004). Protein synthesis is therefore a CPZ target since 30 µM CPZ significantly inhibits translation and higher concentrations (200 µM) completely blocks protein synthesis (De Filippi *et al.,* 2007). It is important to emphasize the fact that CPZ effect on lipids disappears after 24 hours while its effect on proteins is still present which suggests that distinct mechanisms are involved.

In conclusion, CPZ effect on yeast protein and lipid content are in agreement with those reported in animal cell models regarding the involvement of oxidative stress. A CPZ toxic dose causes a decrease of lipid content in yeast which resists the chemical stress after 24 hours. Total proteins are also decreased by CPZ and the effect persists at 24 hours. Mechanisms and actors that mediate the observed CPZ effects, as well as the decreased lipid types remain to be investigated. *Saccharomyces cerevisiae* is a simple not expensive model that is useful to better understand CPZ toxicity and to reduce animal use in experimentation.

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