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Dodecyltriphenylphosphonium inhibits multiple drug resistance in the yeast *Saccharomyces cerevisiae*

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ABSTRACT

Multiple drug resistance pumps are potential drug targets. Here we asked whether the lipophilic cation dodecyltriphenylphosphonium (C_{12}TPP) can interfere with their functioning. First, we found that suppression of ABC transporter gene *PDR5* increases the toxicity of C_{12}TPP in yeast. Second, C_{12}TPP appeared to prevent the efflux of rhodamine 6G – a fluorescent substrate of Pdr5p. Moreover, C_{12}TPP increased the cytostatic effects of some other known Pdr5p substrates. The chemical nature of C_{12}TPP suggests that after Pdr5p-driven extrusion the molecules return to the plasma membrane and then into the cytosol, thus effectively competing with other substrates of the pump.

1. Introduction

As in many cases, pathogenic fungi possess a robust MDR system [1,2], MDR pumps are potential targets of antimycotic mixtures. We decided to test whether dodecyltriphenylphosphonium (C_{12}TPP) and its plastoquinone derivative SkQ1, [3], the nontoxic penetrating lipophilic cations (Fig. 1A), can be used to suppress MDR in yeast cells. Two lines of evidence were pointing in this direction.

First, C_{12}TPP and SkQ1 are very likely to be substrates of MDR pumps in the plasma membrane. Indeed, amphiphilic cations are thought to be preferable substrates of mammalian P-glycoprotein and bacterial ATP binding cassette (ABC) proteins [4]. In particular, it was shown that triphenylmethylphosphonium and tetraphenylphosphonium are substrates of mammalian P-glycoprotein [5]. The fluorescent amphiphilic cation rhodamine 6G was found to be a substrate of yeast Pdr5 – a multidrug pump [6]. The antiarrhythmic drug amiodarone, which is able to inhibit MDR in human cell lines [7], was found to enhance C_{12}TPP-stimulated respiration in MDR-positive but not in MDR-negative yeast cells [8]. Moreover, it was shown that the inhibitor of multidrug efflux pluronic L61 induces accumulation of C_{12}TPP-based antioxidants SkQ1 in K562 myeloid leukemia cells [9]. Not surprisingly, the antioxidant SkQ1 was shown to protect the MDR-negative cells but not the MDR-positive ones against pro-oxidant treatments [10].

Second, being both charged and hydrophobic, the cations are highly membranophilic, i.e. they tend to accumulate at the lipid/water interface. Thus we reasoned that the molecules extruded from the cell may then become immediately trapped in the outer leaflet of the plasma membrane and then move back into the inner one. The reverse transport is expected to be facilitated by the charge: in fungi the outer membranes typically maintain electric potential of up to the 200 mV [11], which acts to attract the penetrating cations into the cells. Therefore, such futile cycling seemed likely to compete with other substrates of MDR pumps.

In this work, using *Saccharomyces cerevisiae* as a model cell system, we found that the ABC-transporter Pdr5p protects the cells from the toxic effects of C_{12}TPP. Consistent with this, we show that C_{12}TPP augments the toxic effects of Pdr5p substrates cycloheximide D and clotrimazole and also inhibits rhodamine 6G efflux.

2. Material and methods

2.1. Strains and growth conditions

In this work we used *W303-1A S. cerevisiae* strains and its derivatives: *AD1-8* with deletions of eight MDR genes [*W303-1A*,

Abbreviations: C_{12}TPP, dodecyltriphenylphosphonium; R6G, rhodamine 6G; MDR, multiple drug resistance; FCCP, carbonylcyanide p-trifluoromethoxyphenylhydrazone; ABC, ATP binding cassette.

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yor1::hisG, snq2::hisG, pdr5::hisG, pdr10::hisG, pdr11::hisG, ycf1::hisG, pdr3::hisG, pdr15::hisG] [12], P_{GAL}-*PDR5* [*W303-1A HIS3::*P_{GAL}-*PDR5*], P_{GAL}-*SNQ2* [*W303-1A HIS3::*P_{GAL}-*SNQ2*], and P_{GAL}-*YOR1* [*W303-1A HIS3::*P_{GAL}-*YOR1*]. Cells were grown in YPD medium (2% glucose, 1% bacto-peptone, 1% yeast extract) or in YPGal (2% galactose, 1% bacto-peptone, 1% yeast extract). For genetic screening and maintaining of strains with conditionally expressed genes, synthetic drop-out media YNB-Leu or YNB-His were used according to Sherman 2000 [13]. The growth rates were measured by increase in light scattering (λ = 550 nm) in liquid yeast culture.

2.2. Microscopy

Cells stained with R6G were visualized with an upright Olympus BX2 microscope and U-MNG2 filter set (excitation 530–550 nm, 570 nm beamsplitter filter, emission >590 nm).

2.3. Genetic screening

Yeast mutants of *W303* strain of *S. cerevisiae* carrying multicopy plasmid YEp13 with inserts of 8–10 Kb at BamHI restriction site were constructed by transformation. Three cycles of enrichment of the mutant collection for C_{12}TPP-resistant strains were performed. During each cycle the mutants at logarithmic stage of growth on YNB-Leu media were treated with 18 μM C_{12}TPP for 3 h, then washed, diluted, and grown overnight on fresh solid YNB-Leu. After the third cycle, the cells were transferred onto solid YNB-Leu media. C_{12}TPP resistance of separated colonies was compared with a wild type. To identify the genes carried by the multicopy plasmid, the genomic DNAs of the selected strains were transformed in *E. coli*. Loci of insertion were determined by sequencing the selected YEp13-insertion plasmids with primers YEp13-DIR 5′-cgctatatgcgttgatgc YEp13-REV 5′-cctgccaccatacccacg.

2.4. Rhodamine 6G efflux

To measure the relative rate of rhodamine 6G efflux, we used the fluorometric assay described by Kolaczkowski et al. [6] with a few modifications. Cells were grown overnight in 40 ml in liquid YPD to the density of 0.5–1 × 10^7 cells/ml, washed twice with cold sterile water, and resuspended in 10 ml phosphate buffer saline supplemented with 5 mM 2-deoxyglucose and 2.5 mM 2,4-dinitrophenol. The cell suspension was incubated for 45 min on a rotatory shaker, and then the inhibitors were removed by two cycles of centrifugation/resuspension in cold water. The energy-deprived cells were resuspended in 10 ml of PBS and then stained with R6G (10 μM) for 40 min. Then the cell suspension was pelleted, resuspended in an equal volume of PBS, and stored on ice for 1–5 h. The efflux was measured with a FluoroMax-3 fluorometer system with excitation wavelength set to 480 nm, and emission wavelength set to 560 nm. The efflux of R6G was initiated by addition of 1% glucose; cell density in the fluorometric cuvette was 10^6 cells/ml.

2.5. Survival assay

Exponentially growing cells were taken and treated with indicated amounts of C_{12}TPP or SKQ1 for 3 h. Then the cell suspensions were plated on solid YPD medium and incubated for 48 h, and the number of formed colonies was counted. 100% refers to the number of colony forming units (CFU) in the yeast suspension at the beginning of the experiment.

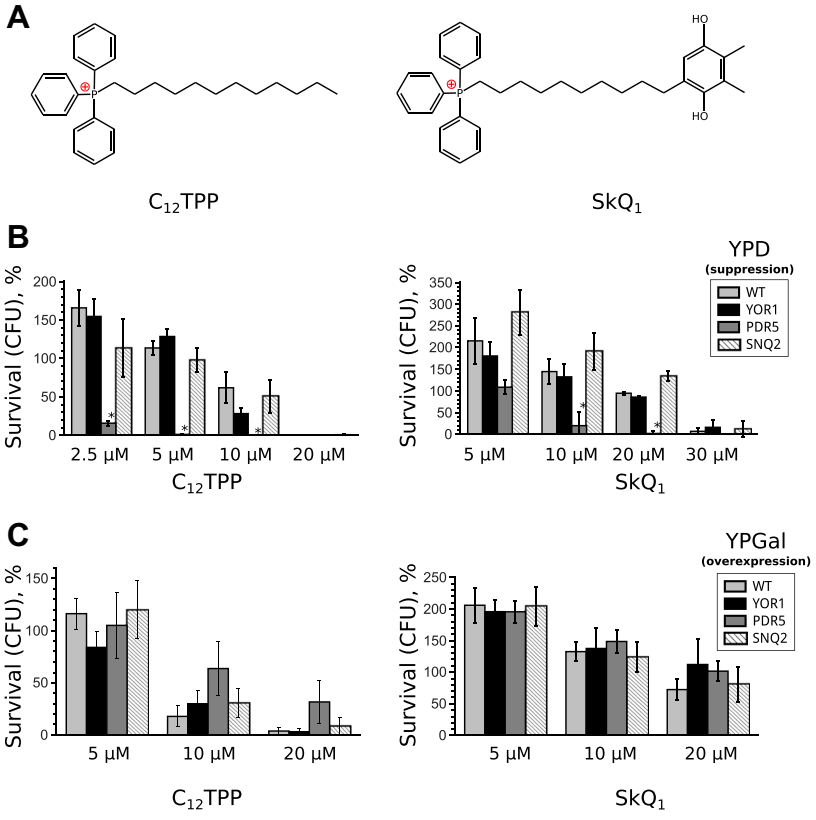

Fig. 1. Expression levels of Pdr5 affect resistances to C_{12}TPP. (A) Chemical structure of dodecyltriphenylphosphonium C_{12}TPP and its plastoquinone derivative (SkQ1) used in this study. Glucose-grown (B) or galactose-grown (C) cells treated with indicated concentration of C_{12}TPP or SkQ1. *$P < 0.05$ compared to untreated wild type (WT) according to Wilcoxon signed-ranked unpaired test.