SELECTION OF RESISTANCE TO CISPLATIN IN *SACCHAROMYCES CEREVISIAE* AS A MODEL FOR ANTINEOPLASTIC DRUGS STUDIES

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Introduction

Resistance of tumour cells to different antineoplastic agents is a major problem in chemotherapy treatment. The spectrum of antineoplastic drugs that can be used is very wide such as methotrexate, cisplatin, mitomycin C, bleomycin, etc. Laboratory studies can evaluate the potency of these drugs by different methods and cellular models. We used cisplatin as a drug model due to its mechanism of action. It forms cytotoxic adducts with the DNA dinucleotide d(pGpG), inducing intra-strand crosslinks, leading to apoptosis. The mechanisms involved in cisplatin resistance include the decrease of the cellular entry of the drug and increase of the extrusion, activation of detoxification systems, alteration of cell targets, increase in DNA repair, alteration of apoptosis and alteration of the oncogenes expression. The budding yeast (*S. cerevisiae*) is an excellent eukaryotic model to study the antineoplastic drugs effects, due to the well-characterized metabolic and genetic characteristics and the conserved similarity in molecular mechanisms with other species including human cells.

Objectives

The aim of this work is to study the effect of cisplatin on *S. cerevisiae* cells and select cisplatin-resistant cells of this organism as a model of study of resistance to antineoplastic drugs.

Methodology

Yeast strain and culture medium

The experiments were carried out with the haploid yeast strain *S. cerevisiae* WS8105-1C (genotype: MATalpha, ade2, arg4-17, trp1-289, ura3-52). Yeast cells were grown in a solid medium of YPD for the cytotoxicity assay, and in a liquid medium of YPD for selecting cisplatin-resistant cells of this organism.

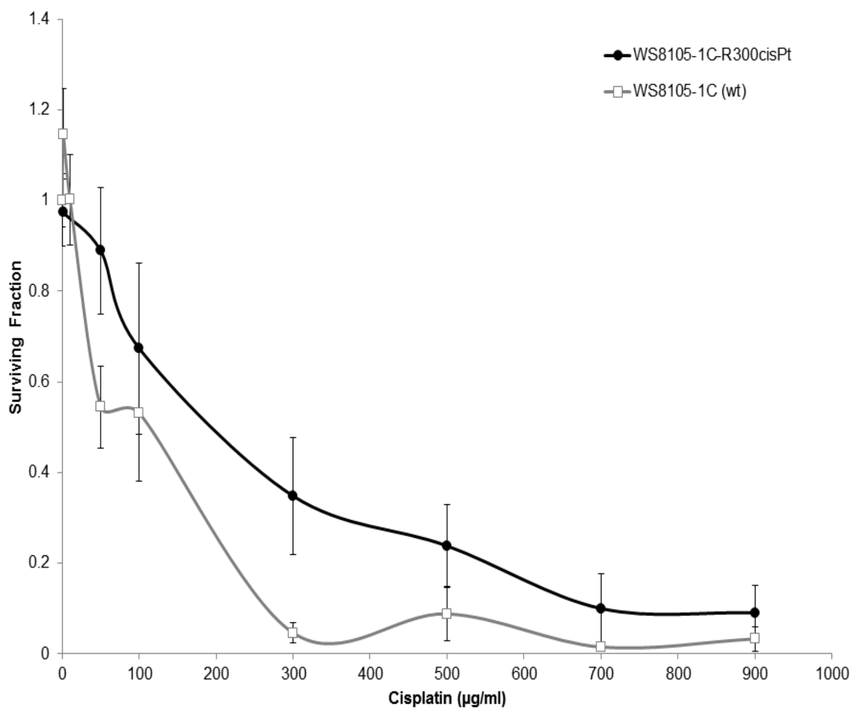
Chemicals

The antineoplastic drug used was cisplatin. The doses used were 0, 50, 100, 300, 500, 700 and 900 µg/ml for the cytotoxicity assay and 300 µg/ml for selecting cisplatin-resistant cells.

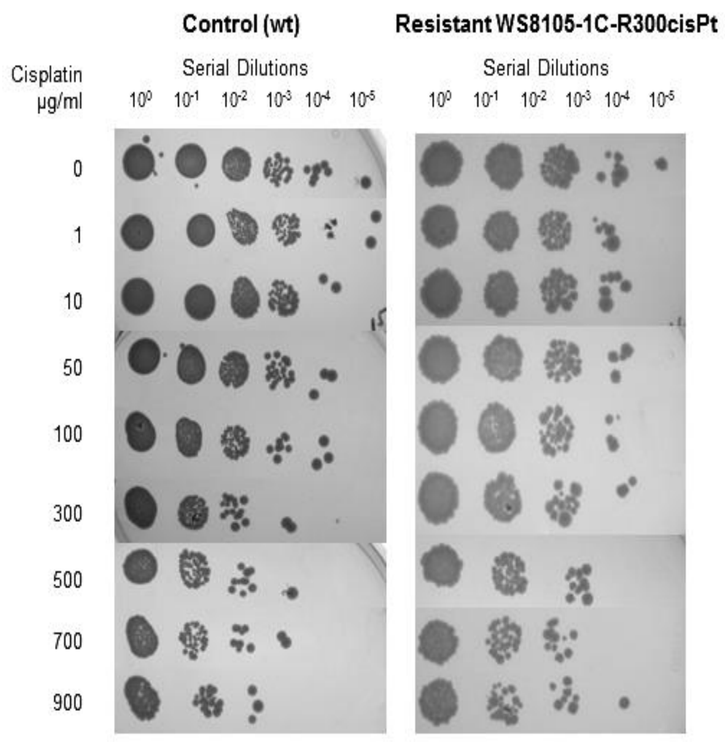
Experimental protocol

Cytotoxicity test: cells were added to test tubes with different doses of cisplatin and they were completed with sterile water. Then, the tubes were cultured during 24 hours at 30°C and cells washed twice with sterile water. For drop test assay, six 10-fold serial dilutions from each sample were prepared and five-microliter aliquots of each dilution were spotted onto YPD plates. The same test was carried out to the resistant strain WS8105-1C-R300cisPt.

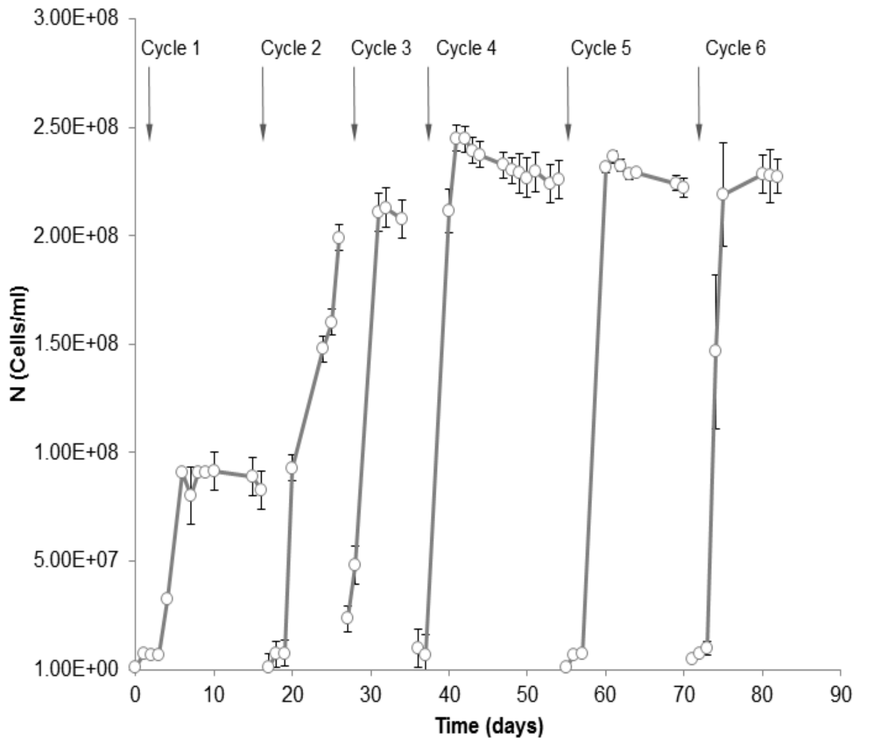
Resistance selection: cells were added to test flask with 8 ml of liquid medium of YPD and 300 µg/ml of cisplatin. Then, the flask were cultured during 10 days in orbital shaking at 300 rpm and at 30°C. Next, a reseeding was carried out in another test flask with the same conditions. The process was carried out six times (six cycles). The OD600 was measured daily.



Evaluation of Cisplatint cytotoxicity. wt: wild strain (control). WS8105-1CR300cisPt: cisplatin-resistant strain. Mean ± SD. p = 0.005; (ANOVA).



Cisplatin cytotoxicity evaluated by drop test. wt: wild strain (control). WS8105-1C-R300cisPt: Cisplatin resistant strain.



Temporal evolution of the Cisplatin resistant selection process. Mean ± SD.

Results and conclusions

TheThe cytotoxicity curve for cisplatin obtained by drop test showed that the surviving fraction decreased gradually as cisplatin dose increased in the wild strain *S. cerevisiae* (WT). The ID50 and ID90 values obtained were 90 µg/ml and 300 µg/ml, respectively.

When exposed the WT to 300 µg/ml of cisplatin, in the different resistance cycles, it was obtained that in cycle 1 and for a N value of 9x107 cells/ml the stationary phase was reached on day 6. However, for cycles 3-6 and for an average value of N of 2.25x108 cells/ml the stationary phase was reached around day 4.

In addition, there was a delay in cell growth. So, for a value of N of 9x107 cells/ml cycle1 presented a time of 6 days, cycle 2 of 3 days, cycle 3 and 4 of 1.75 days, cycle 5 of 3 days, and cycle 6 of 2.5 days. Thus it was observed that for the same value of N, as more cycles were done, the time to reach that value decreased.

The 6 cycles lasted 80 days. The most relevant were cycle 1, which for a N value of 9x107 cells/ml presented a time of 6 days, cycle 3 that for a N value of 2.1x108 cells/ml presented a time of 30 days and cycle 4 that for a N value of 2.45 x108 cells/ml it presented a time of 40 days.

To confirm that the yeast acquired resistance, the same cytotoxicity test was carried out. The decrease of the surviving fraction in the WT was clearly greater than in strain WS8105-1CR300cisPt (resistant strain). In addition, in the resistant strain the ID50 and ID90 values obtained were 224 µg/ml (2.5 times more resistant with respect to the WT) and 735 µg/ml (2.78 times more resistant with respect to the WT), respectively.

In conclusion, at first cells were sensitive to the drug and had low cell growth, but when cells were subjected to drug concentration in each cycle, they developed resistance mechanisms that allowed them to survive, grow and divide in the presence of cisplatin, thus increasing cell growth. In addition, it could also be observed that the stationary phase was reached in a shorter time. These facts indicate the acquisition of resistance.

Therefore, the present results and model could be useful for future antineoplastic drugs studies.