IN VITRO CISPLATIN CYTOTOXICITY IN THE TESTICULAR GERM TUMOR CELL LINE NTERA-2IS ENHANCED BY THE CDK4/6INHIBITOR PALBOCICLIB

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Introduction: Testicular germ cell tumors (TGCTs) are the most common type of cancer in men between 20 and 40 years of age. Cisplatin-based chemotherapy is the mainstay in the treatment of TGCTs and about 70–80% of the patients with disseminated testicular cancer can be cured with this treatment. Unfortunately, 20–30% of patients do not respond, or relapse. Because of not satisfying results in the treatment of relapsed TGCTs, evaluation of new treat-ment strategies and novel drugs with significant antitumor activity, as a single-agent or combi-nation, is a priority.

Methods: Cell culture and treatment. Ntera-2Clone D1was purchased from ATCC® and cul-tured as suggested. Cells (2.0 x 104/well in 24wells-plate) were treated with increasing concen-trations of cisplatin (range: 0.001- 10 μM) and/or palbociclib (range: 0.5- 10 μM). Preliminary experiments of time-course have been conducted to establish the optimal duration of cell treatment. To evaluate the duration of cytotoxicity after drug suspension, cells were treated for 2days with the IC50 value of cisplatin and/or palbociclib, then the medium was replaced with drug-free medium and cells were kept in culture up to 12days. Cell viability was evaluated by 3-(4,5-Dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye reduction assay and/or by ATP-Lite Luminescence Assay (PerkinElmer, Milan, Italy). Each experiment was per-formed at least 3times and run in triplicate. For the evaluation of drug combination, the Chou-Talaly method was applied and the isobologram-multiple drug effect equation was determined using the CompuSyn software. Gene and protein expression were evaluated respectively by q-RT-PCR (ViiA7, Applied Biosystem, Milan, Italy), using the SYBR Green as fluorochrome; and by western blot, using the 4-12% NuPAGE bis-tris gel system (Life Technologies, Milan, Italy). Den-sitometric analysis of the immunoblots was performed using ImageJ software. Data analysis were conducted with the Graph Pad Prism 5software.

Results: Ntera-2cell line exposed to increasing concentrations of cisplatin displayed a concen-tration-dependent cytotoxicity, that reached its maximum at 48hours of treatment, with the IC50 of 0.3μM. Cisplatin exposure induced an increase of both RNA and protein of cdk6, with no modification of cdk4expression. Based on this results, Ntera-2cells were exposed to increasing concentrations of the cdk4/6inhibitor palbociclib, inducing a cytotoxic effect, with the IC50 of 2.3μM. Interestingly, the combination experiments applying the Chou-Talalay method, indicated that a synergism can be observed when cells are exposed to both drugs. The drug combination ex-erted a positive effect also for the cell recovery after the toxic insult elicited by these anti-cancer drugs: indeed, when cells were exposed to both drugs at their IC50, the latency of cell proliferation recovery lasted up to 8days and cells returned to the untreated cell proliferation rate 10 days after drug withdrawal, while the latency was inferior (up to 5days) when cells were treated with cisplatin or palbociclib alone. To evaluate palbociclib activity, gene and protein expression of cdk4/6and their downstream targets Rb/pRb were measured. Accordingly to the palbociclib mechanism of action, pRb was reduced in Ntera-2cells after palbociclib treatment, while no modifications were detected in gene expression of cdk4/6, while an increase of protein expression was observed. Mechanism involving this phenomenon are now under investigation.

Conclusions: The cytotoxic effect of cisplatin in Ntera-2cells was increased when the drug was combined with the cdk4/6inhibitor palbociclib. Accordingly, palbociclib is currently used as com-bination therapy in the breast cancer therapy, in association with aromatase inhibitors or fulves-trant. These results could give the background and the rationale to develop further studies in order to improve the pharmacological therapy of testicular cancer.