IN VITRO ASSESSMENT OF CABAZITAXEL ACTIVITY IN ADRENOCORTICAL CARCINOMA CELLS

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Adrenocortical cancer (ACC) is a rare aggressive disease with a poor prognosis (1). The treatment of metastatic ACC is challenging and the current available treatments are mitotane, chemotherapy or the combination of both (2); however, prognosis in locally advanced inoperable and metastatic ACC patients still remains poor. New treatment strategies are therefore needed. One of the most important mechanisms limiting the efficacy of many chemotherapeutic drugs in ACC is the high cellular expression of the MDR-1 gene, that encodes for the P-glycoprotein (Pgp), responsible of the multidrug resistance (3). Cabazitaxel is a novel taxane with a poor affinity for Pgp; indeed, preclinical data showed its ability to inhibit cell growth in a wide range of human cancer cell lines expressing MDR-1 (4).

In the present study, we investigate the in vitro activity of cabazitaxel in the experimental model of the human ACC derived NCI-H295R cell line. Cabazitaxel displayed improved potency and efficacy compared to the other clinically used taxanes, namely docetaxel and paclitaxel. In particular, NCI-H295R cells were exposed to increasing concentrations of each taxane (0.1-500 nM) for 4 days and analyzed for cell viability by using MTT assay. The calculated IC50 for each drug revealed an order of potency that was: cabazitaxel (13.3 nM, IC95%: 9–19.6 nM) > docetaxel (38.7 nM, IC95%: 20-74.3 nM) > paclitaxel (84.3 nM, IC95%: 43.5-163.5 nM), although differences did not reach a statistical significance. The efficacy improvement is strongly suggested by our results showing that the inhibition of cell viability was present already after 1 day of treatment and that it was significantly higher compared to docetaxel and palcitaxel. It should be underlined as well that the inhibition of NCI-H295R cell viability was maintained up to 4 days of treatment, with a high linear correlation (regression coefficient r2 =0.97). The IC50 value in the range of low nM concentration suggested a high affinity for the microtubule β-tubulin subunit. Like most other microtubule inhibitors, cabazitaxel induced classical apoptotic cell death through intrinsic pathway (5) in ACC cells, as we observed using the Human Apoptosis Array screening approach. Cabazitaxel induced apoptosis, reflected by different regulation of several protein, including member of the bcl-2 family, cleaved-caspase 3 and proteins involved in cell cycle regulation. NCI-H295R cells expressed high level of both mRNA and protein of Pgp, analyzed by qRT-PCR and Western Blot. To gain insight into the role of MDR1 in ACC cell sensitivity to taxanes, the siRNA approach was used to knockdown MDR1. We observed that MDR1 silencing increased the cytotoxic effect of docetaxel and paclitaxel in NCI-H295R cells compared to untreated cells, but, as expected, no difference was observed in cells exposed to cabazitaxel. The antitumor effect of cabazitaxel was also evident in three primary cell cultures: ACC03, ACC08 and ACC16, established from patients underwent surgery for ACC, with a cytotoxic effect that reached its maximum of 63.2 ± 0.9 % after 4 days of treatment, with an IC50 value of 3.2 nM, (IC95%: 2.5–4.1 nM), 1.1 nM, (IC95%: 0.9–1.4 nM) and 16.5 nM, (IC95%: 10.2–26.7 nM) respectively.

Taken together, these in vitro data indicate that cabazitaxel displayed a good efficacy profile that has led to the design of a clinical study (EUDRACT 2017-001591-35) in patients with advanced ACC progressing after previous chemotherapy lines.

References

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