Gynecologic Oncology 128 (2013) 377–382

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Navitoclax augments the activity of carboplatin and paclitaxel combinations in ovarian cancer cells

Vasileios A. Stamelos [a], Elizabeth Robinson [a], Charles W. Redman [c], Alan Richardson [a,b,*]

[a] Institute for Science and Technology in Medicine, Guy Hilton Research Centre, Keele University, Thornburrow Drive, Stoke-on-Trent, ST4 7QB, UK
[b] School of Pharmacy, Keele University, Keele, Staffordshire, ST5 5BG, UK
[c] University Hospital of North Staffordshire, Newcastle Road, Stoke on Trent, Staffordshire, ST4 6QG, UK

HIGHLIGHTS

- Navitoclax combined with either carboplatin or paclitaxel inhibits cell growth more effectively than carboplatin and paclitaxel doublet combinations.
- In triplet combinations, navitoclax reduces the antagonism between carboplatin and paclitaxel.
- Navitoclax augments the activity of carboplatin and/or paclitaxel in Igrov-1 spheroids.

ARTICLE INFO

Article history:
Received 21 September 2012
Accepted 14 November 2012
Available online 17 November 2012

Keywords:
Ovarian
Carboplatin
Navitoclax
Paclitaxel
Resistance

ABSTRACT

Objectives. To evaluate the efficacy of combination of navitoclax, carboplatin and paclitaxel in ovarian cancer.

Methods. 8 ovarian cancer cell lines were treated with either doublet or triplet combinations of navitoclax, carboplatin and paclitaxel. Interactions were assessed by determining a combination index or measuring caspase activity. The effect of the combinations was also evaluated by measuring the inhibition of cells grown as spheroids.

Results. Navitoclax exhibited modest (IC_{50} = 3–8 μM) single agent potency. Antagonism between carboplatin and paclitaxel was evident in Ovcar-4, Ovcar-8 and Skov-3 cells. Drug combinations including navitoclax with carboplatin and/or paclitaxel showed significantly less antagonism, or even synergy, in several cell lines than carboplatin/paclitaxel alone. Navitoclax enhanced the activation of caspase 3/7 induced by carboplatin and/or paclitaxel in Igrov-1 cells. Combinations of navitoclax, carboplatin and paclitaxel showed more than additive activity against Igrov-1 spheroids.

Conclusions. Navitoclax improves the activity of combinations of carboplatin and paclitaxel *in vitro*. Our observations, taken with other published data, provide a rationale for clinical trials of navitoclax in ovarian cancer in combination with chemotherapy.

Introduction

Ovarian cancer represents an unsatisfied medical need. Although many patients respond well to surgical debulking and first line chemotherapy, the emergence of drug resistance hampers long term survival [1]. One strategy to identify novel treatments is to identify drugs which reduce the resistance of cancer cells to carboplatin and paclitaxel, the chemotherapeutic drug combination most commonly used to treat ovarian cancer.

Many chemotherapeutic agents trigger apoptosis by activation of either the intrinsic or extrinsic apoptosis pathway. In the case of the intrinsic pathway, DNA damage induced by chemotherapy elicits the production of pro-apoptotic proteins that can cause mitochondrial permeabilization. This process is regulated by Bcl-2 family proteins that either inhibit or promote apoptosis [2]. Bax and Bak are apoptosis effectors which form a pore in mitochondrial outer membrane and commit cells to undergo apoptosis. These proteins are inhibited by the multi-domain Bcl-2 family apoptosis inhibitors which bind the BH3 domain in Bak and Bax. BH3-only proteins are pro-apoptotic proteins which occupy the apoptosis inhibitors preventing them from sequestering Bax or Bak. Some BH3-only proteins may also directly activate Bak and Bax. Drugs termed "BH3 mimetics" have recently been developed which antagonize the Bcl-2 family apoptosis inhibitors [3] and diminish the capacity of the inhibitors to sequester the pro-apoptotic proteins. This effectively lowers the threshold at which cells undergo apoptosis. BH3 mimetics are not thought to induce directly apoptosis on their own, but require the induction of the pro-apoptotic BH3 only proteins. Consequently, BH3 mimetics have shown synergy with several chemotherapeutic agents that induce BH3-only proteins [4,5].

* Corresponding author at: Institute for Science and Technology in Medicine, Guy Hilton Research Centre, Keele University, Thornburrow Drive, Stoke-on-Trent, ST4 7QB, UK.
E-mail address: a.richardson1@keele.ac.uk (A. Richardson).

0090-8258/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygyno.2012.11.019

One BH3 mimetic that has been widely studied is ABT-737 [4]. Although ABT-737 has not entered clinical trials because of its poor oral bioavailability, a closely related drug, ABT-263 [6] ("navitoclax") is currently undergoing clinical evaluation. Encouraging initial results have been observed in a range of lymphoid malignancies [7], and in a recent phase 1 trial, 35% of patients achieved a partial response [8]. Unfortunately, limited single agent activity was observed in patients with relapsed small cell lung cancer [9]. The presence of pre-existing complexes of anti-apoptotic proteins primed [10] with pro-apoptotic proteins has justified the use of navitoclax as a single agent; navitoclax (or ABT-737) liberates the pro-apoptotic proteins and induces apoptosis [10]. However, in other cancer types, the lack of these "primed" complexes suggests the evaluation of navitoclax in combination with other pro-apoptotic stimuli, such as chemotherapy, to induce pro-apoptotic Bcl-2 family proteins. In clinical trials this could be achieved by including a BH3 mimetic with existing chemotherapy.

We have previously shown that the sensitivity of ovarian cancer cells to carboplatin can be increased by ABT-737 [11]. ABT-737 showed only modest single agent activity against the same cells in culture, although activity both as a single agent and in combination with carboplatin was observed in xenograft studies. This raised the potential for the clinical evaluation of navitoclax with carboplatin in the treatment of ovarian cancer. However, both carboplatin [12] and navitoclax [13–15] induce thrombocytopenia, raising concerns about the potential clinical toxicity of this drug combination. Paclitaxel is known to reduce the impact of carboplatin on platelets in patients receiving carboplatin [16,17], making it desirable to consider inclusion of paclitaxel with combinations of carboplatin and navitoclax. However, in our original studies, we observed additivity between paclitaxel and ABT-737, although others have recently reported synergy between paclitaxel and navitoclax in ovarian cancer cells [18]. In addition, numerous studies have pointed to antagonism between paclitaxel and platinum-based therapeutics in cell proliferation studies *in vitro* [19–23]. These observations raised the question whether paclitaxel might interfere with synergy between navitoclax and carboplatin. We therefore wished to evaluate the efficacy of combinations of carboplatin, paclitaxel and navitoclax. Our observations demonstrate that navitoclax augments the activity of either carboplatin, or paclitaxel or the carboplatin–paclitaxel combination in ovarian cancer cells.

Methods

Navitoclax (Chemitek) and paclitaxel (Sigma) were dissolved in DMSO and carboplatin (Sigma) was dissolved in PBS. Cells were cultured in RPMI supplemented with 10% FCS, 50 U/ml penicillin/streptomycin and 2 mM glutamine. Cultures of Ovcar-3 cells were additionally supplemented with 0.01 mg/ml insulin and 1 mM sodium pyruvate.

Cell growth assays with monolayers were performed by incubating cells with the indicated drugs for 72 h as previously described and the surviving cells were estimated by staining with sulforhodamine B [11]. For each single agent, double or triple drug combination, complete dose–response curves were determined in every experiment, using 18 different drug concentrations to establish IC_{50} values and Hill coefficients. For drug combinations, carboplatin and paclitaxel were combined at the ratio of their IC_{50}s. Where navitoclax was included, a single fixed concentration, estimated from preliminary IC_{50} determinations on its own to inhibit proliferation by 5%, was used while the concentration of the chemotherapeutic agent was varied. The fixed concentrations of navitoclax were: A2780, 3.3 μM; cisA2780, 1.5 μM; Ovcar-3, 1.2 μM; Ovcar-4, 2.1 μM; Ovcar-5, 0.7 μM; Ovcar-8, 1.5 μM; Igrov-1, 0.4 μM; and SkOv-3, 0.6 μM. Using a fixed concentration overcomes problems associated with drug insolubility that can occur at high concentrations if a drug that has a high IC_{50} is included in combination at a fixed ratio [11]. Data were analyzed using Graphpad Prism to fit a four parameter Hill equation using non-linear regression. Combination indices (CI) were calculated as described [24] and are quoted at fraction affected = 0.5. We considered drug interactions to be either additive (CI = 1) unless the combination index differed significantly from unity and interactions were then considered synergistic (CI < 1.0) or antagonistic (CI > 1).

To measure caspase 3/7 activity, 5000 cells were seeded per well of a 96 well plate. The following day cells were exposed to the indicated concentration of drug. After 30 h, caspase activity was measured using Caspase 3/7 Glo (Promega) according to the manufacturer's instructions.

To measure the activity of drugs using spheroids, cells were resuspended in complete medium (2×10^6 cells/ml) and 20 μl pipetted on the up-turned lid of a 48 well plate. Only the inner 'wells' of the lid were used and 20 μl of medium was placed in the remaining lid 'wells'. 300 μl of PBS was placed in the well beneath the lid to ensure a humid atmosphere. After 1 week, the spheroids were exposed to drug by addition of 5 μl of RPMI containing the drug or solvent. After a further 72 h, the spheroids were collected with a wide bore pipette into a 96 well plate and 25 μl of Cell-titre Glo (Promega) reagent was added to measure ATP. Microscopic inspection confirmed that this method fully lysed the spheroids, avoiding issues of the reagent penetration into the spheroid affecting the measurement. For IC_{50} measurements, the data were analyzed using Graphpad Prism to fit a four parameter Hill equation using non-linear regression. To compare the observed effect of the drug combinations in these experiments with the expected effect, the Bliss independence criterion was used to calculate the expected effect of the combination from the effect of the individual single agents as described [25].

Results

Prior to evaluating the combination of carboplatin, paclitaxel and navitoclax, we first measured the potency of these drugs as single agents. We have previously reported the sensitivity of the cell lines used in this study to carboplatin or paclitaxel [11] and comparable values were obtained in these experiments. Navitoclax as a single agent inhibited the growth of all 8 cell lines with potencies (IC_{50} = 3–8 μM; Table 1) that were slightly more potent (approximately 2-fold) than we had previously observed with ABT-737 [11].

We next measured the effect of each combination of two of the three drugs under evaluation. In these experiments we used navitoclax at a fixed concentration that is comparable to the levels observed in phase 1 clinical trials with navitoclax [14,26]. In 4 of 8 cell lines evaluated, synergy between navitoclax and carboplatin was observed (CI < 1; Fig 1A). This was most pronounced in Igrov-1 cells, the cell line in which synergy between carboplatin and ABT-737 was most evident in our previous studies with ABT-737 [11]. We also observed antagonism between carboplatin and navitoclax in the A2780 cell line and its platinum-resistant derivative cisA2780.

Although carboplatin and paclitaxel are used in combination in first line therapy for ovarian cancer, a number of reports have previously described antagonism between these two agents [19,23]. Consistent

Table 1
Single agent potency of navitoclax. Cells were treated with a range of concentrations of navitoclax for 72 h and stained with SRB, and the IC_{50} (mean ± S.D.) was calculated from the indicated number (n) of experiments.

Cell line	IC_{50} (μM)	n
A2780	6.8 ± 2.5	9
cisA2780	4.7 ± 2.0	5
Ovcar-3	5.9 ± 0.4	3
Ovcar-4	5.9 ± 3.5	6
Ovcar-5	3.2 ± 1.3	10
Ovcar-8	5.3 ± 1.5	13
Igrov-1	8.5 ± 5.7	16
Skov-3	5.2 ± 1.5	6