NOTE

PARP Inhibitors as P-glyoprotein Substrates

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ABSTRACT: The cytotoxicity of PARP inhibitors olaparib, veliparib, and CEP-8983 were investigated in two P-glycoprotein (P-gp) overexpressing drug-resistant cell models (IGROVCDDP and KB-8-5-11). IGROVCDDP and KB-8-5-11 were both resistant to olaparib and resistance was reversible with the P-gp inhibitors elacridar, zosuquidar, and valspodar. In contrast, the P-gp overexpressing models were not resistant to veliparib or CEP-8983. Olaparib and veliparib did not induce protein expression of P-gp in IGROVCDDP or KB-8-5-11 at doses that successfully inhibit PARP. Olaparib therefore appears to be a P-gp substrate. Veliparib and CEP-8983 do not appear to be substrates. Veliparib and CEP-8983 may therefore be more useful in combined chemotherapy regimens with P-gp substrates and may be active in platinum and taxane-resistant ovarian cancer. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: olaparib; veliparib; CEP-8983; PARP inhibitor; drug Resistance; cell lines; P-glycoprotein; cancer chemotherapy; toxicity

INTRODUCTION

Parp inhibitors are a new class of chemotherapy agents that target the cell's DNA damage repair pathways. PARP inhibitors are potentially very useful for treating BRCA1/2-dysfunctional cancers, as in these cancers the DNA repair machinery is already impaired. The results of proof of concept clinical trials of the PARP inhibitor olaparib in breast and ovarian cancer patients with germline BRCA1/2 mutations have been encouraging.[1,2]

For any new chemotherapy agents, it is important to establish if they are substrates of the classical ABC transporters, such as P-glycoprotein (P-gp). Agents that are not P-gp substrates may be more useful clinically, as if transporter-driven drug resistance develops the cells are unlikely to be resistant to the wide range of chemotherapy drugs that are also P-gp

substrates. P-gp mRNA has been detected in primary ovarian tumors,[3] and its expression has been associated with poor overall survival.[3] Between 16-25% of primary ovarian tumors are highly positive for P-gp by immunohistochemistry (IHC).[4–6] There is limited clinical data to support the induction of P-gp in the clinic, unlike in cancer cell lines treated with chemotherapy. Despite this, some studies have shown P-gp staining to increase in ovarian tumors after chemotherapy.[6] P-gp has been shown to be an independent prognostic factor in some ovarian cancer studies[4] but not in others.[5,6] Similarly, between 44% and 66%[7,8] of breast cancers stain positive for P-gp by IHC, some studies found it to be an independent prognostic factor[7] and others did not.[8] The induction of P-gp in response to doxorubicin and epirubicin treatment was found to be predictive of survival in one breast cancer study.[9] The role of P-gp in BRCA1-mutated clinical breast or ovarian cancer has not been studied in detail. However, a study that examined the gene expression profiles of BRCA1/2 tumors ($n = 34$) versus sporadic ovarian cancer ($n = 27$) in an Ashkenazi Jewish population did not find P-gp to be significantly differentially expressed.[10]

There is currently limited data on the P-gp substrate status of PARP inhibitors. Olaparib has been shown to induce P-gp gene expression in an animal tumor model.[11] Veliparib has been described as a weak P-gp substrate in a study using a P-gp transfected cell line.[12] In contrast, the novel PARP inhibitor CEP-8983 has not been examined for its P-gp substrate status. There has also been no work to date examining PARP inhibitors using cell models of acquired drug resistance overexpressing P-gp. This study will examine the PARP inhibitors olaparib, veliparib, and CEP-8983 in two cell models of acquired drug resistance where the major mechanism of drug resistance is overexpression of P-gp: IGROVCDDP ovarian cells[13] and KB-8-5-11 cervical cells.[14,15]

MATERIALS AND METHODS

Cell Culture and Cytotoxicity Assays

IGROV-1 and IGROVCDDP ovarian cancer cells[16,17] were obtained from Prof. Jan Schellens (Netherlands Cancer Institute) and grown as previously described.[13] KB-3-1 and KB-8-5-11 cervical cancer cells[14,15] were obtained from Prof. Michael Gottesman (National Cancer Institute) and grown in Dulbecco's modified Eagle's medium (Sigma, Dublin, Ireland), 1% Pen strep, 2% L-glutamine, and 1% Na Pyruvate with 10% FCS (Lonza, Verviers, Belgium). KB-8-5-11 cells were routinely grown in with colchicine; the drug was removed 3 days before the start of all experiments. All cell lines were maintained in a humidified atmosphere with 5% CO2 at 37°C. All cultures were tested routinely and were mycoplasma free. All cell lines were STR fingerprinted to confirm identity.

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PARP inhibitors olaparib and veliparib and zosuquidar were obtained from Selleck chemicals (Houston, TX). CEP-8983 was obtained from Cephalon Inc (Now part of Teva Pharmaceutical industries, Tikva, Isreal). Elacridar was obtained from Santa Cruz Biotechnology (Heidelberg, Germany). Valspodar was obtained from Sigma. To determine the cytotoxicity of the chemotherapy drugs, cells were plated into flat-bottomed, 96-well plates at a cell density of 2×10^4 cells/well and allowed to attach overnight. Twenty-four hours later, wells were treated in triplicate with serial dilutions of drug in a final volume of 200 μL. The concentration ranges of chemotherapy drugs and P-gp inhibitors used for the cytotoxicity assays used on each cell line is given in Table S1. Drug-free controls were included in each assay. Plates were incubated for a further 5 days at 37°C in a humidified atmosphere with 5% CO_2 and cell viability was determined using an acid phosphatase assay for IGROV-1, IGROVCDDP and an MTT assay for KB-3-1 and KB-8-5-11.[18] The MTT assay was used for KB-3-1 and KB-8-5-11 as these cell lines have a low level of acid phosphatase yielding a low absorbance with confluent cells. Similarly, the acid phosphatase assay was used for IGROV-1 and IGROVCDDP as low absorbances were obtained on confluent cells with the MTT assay.

Western Blots

The Western blots were performed as previously described.[13] Primary and secondary antibodies used are listed in Table S2.

Taqman Low-Density Arrays

The Taqman low-density arrays were performed as previously described.[13]

Statistical Analysis

All experiments were performed at minimum in biological triplicate. Two-sample, two-tailed Student's *t*-tests were used to determine significant differences using $p \leq 0.05$ as a cut off.

RESULTS

IGROVCDDP and KB-8-5-11 Are Resistant to Known P-gp Substrates

IGROVCDDP and KB-8-5-11 cells were resistant to known P-gp substrates doxorubicin and vincristine (Tables 1 and 2).[19] The resistance to these agents was reversed in both cell lines by treatment with P-gp inhibitors elacridar,[20] zosuquidar, and valspodar[21] ($p < 0.05$). The dose of 0.25 μM elacridar has been previously shown to prevent P-gp transport activity in IGROVCDDP[13] and KB-8-5-11 cells[22] and has a minimal growth inhibitory effect. The doses of zosuquidar (1.5 μM) and valspodar (0.25 μM IGROV-1 and IGROVCDDP; 31.25 nM KB-3-1 and KB-8-5-11) were optimized to have a minimal growth inhibitory effect on the cell lines while reversing the known P-gp substrate doxorubicin. Zosuquidar used at 1–3 μM has been previously shown in the literature to specifically reverse P-gp transport activity in a variety of cell models.[23,24] Similarly,

Table 1. Resistance Profile of IGROVCDDP Examining P-gp Substrates

	IGROV-1 IC_{50}		IGROVCDDP IC_{50}		Resistant Versus Sensitive		IGROV-1 +/− Inhibitor	IGROVCDDP +/− Inhibitor
Drug (Units)	Mean ± SD	*n*	Mean ± SD	*n*	Fold	*p* Value	*p* Value	*p* Value
Known P-gp Substrates								
Doxorubicin (nM)	21.81 ± 3.73	4	86.04 ± 16.18	4	3.94	2.45E-04		
+ Elacridar 0.25 μM	12.80 ± 0.77	3	12.97 ± 0.92	4	1.01	0.81	0.01	6.07E-04
+ Zosuquidar 1.5 μM	12.52 ± 2.20	3	8.00 ± 1.27	4	0.64	0.02	0.01	7.24E-05
+ Valspodar 0.25 μM	13.92 ± 2.67	5	13.49 ± 1.18	4	0.97	0.80	7.71E-03	1.09E-04
Vincristine (nM)	8.30 ± 1.50	4	26.76 ± 4.24	4	3.22	1.76E-04		
+ Elacridar 0.25 μM	1.69 ± 0.14	4	0.26 ± 0.04	5	0.16	1.14E-07	1.21E-04	1.60E-05
+ Zosuquidar 1.5 μM	1.35 ± 0.12	4	0.27 ± 0.04	5	0.20	2.63E-06	9.03E-05	1.60E-05
+ Valspodar 0.25 μM	1.48 ± 0.20	4	0.55 ± 0.09	3	0.37	6.59E-04	1.04E-04	1.38E-04
Parp Inhibitors								
Olaparib (μM)	1.25 ± 0.11	7	11.17 ± 1.98	8	8.96	6.88E-09		
+ Elacridar 0.25 μM	1.17 ± 0.11	4	4.65 ± 0.49	5	3.99	2.40E-06	0.27	2.30E-04
+ Zosuquidar 1.5 μM	1.90 ± 0.31	5	4.63 ± 0.52	5	2.43	7.81E-06	1.47E-03	6.33E-05
+ Valspodar 0.25 μM	1.45 ± 0.22	5	8.06 ± 1.66	4	5.56	2.14E-05	0.06	0.02
Veliparib (μM)	54.23 ± 5.38	7	50.55 ± 8.33	9	0.93	0.328		
+ Elacridar 0.25 μM	45.88 ± 4.14	7	46.19 ± 7.83	10	1.01	0.926	6.92E-03	6.33E-02
+ Zosuquidar 1.5 μM	44.34 ± 1.60	5	48.77 ± 3.42	8	1.10	0.021	2.78E-03	0.58
+ Valspodar 0.25 μM	38.38 ± 3.41	4	47.13 ± 3.16	7	1.23	0.002	5.25E-04	0.32
CEP-8983 (μM)	5.69 ± 0.75	8	5.35 ± 0.75	8	0.94	0.372		
+ Elacridar 0.25 μM	5.97 ± 1.07	9	5.48 ± 0.78	8	0.92	0.306	0.55	0.73
+ Zosuquidar 1.5 μM	5.14 ± 0.81	6	4.09 ± 0.60	4	0.80	0.134	0.21	0.01
+ Valspodar 0.25 μM	4.45 ± 0.42	5	4.31 ± 0.61	5	0.97	0.692	0.01	0.03
P-gp Inhibitors								
Elacridar (μM)	3.17 ± 0.12	4	1.62 ± 0.03	4	0.51	1.97E-06		
Zosuquidar (μM)	5.81 ± 0.64	4	5.72 ± 1.31	6	0.98	0.90		
Valspodar (μM)	4.15 ± 1.01	4	2.77 ± 0.71	6	0.67	0.03		

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