Brief Report

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Development of a simple high-performance liquid chromatography-ultraviolet detection method for olaparib in patients with ovarian cancer

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SUMMARY Olaparib is a small-molecule inhibitor of poly(ADP)-ribose polymerase (PARP) used as maintenance therapy for recurrent ovarian cancer and newly diagnosed advanced ovarian cancer after initial chemotherapy. An exposure-toxicity correlation has been reported between the probability of anemia, a common adverse event associated with olaparib, and the steady-state minimum plasma concentration (Cmin) as well as the predicted maximum plasma concentration (Cmax). On the other hand, olaparib exhibits high interpatient variability with regard to the area under the concentration-time curve, Cmax, and Cmin. Therefore, we developed a simple and sensitive assay based on high-performance liquid chromatography with ultraviolet light (HPLC-UV) for the therapeutic drug monitoring of olaparib. The analysis was performed on an octadecylsilyl column with a mobile phase consisting of 0.5% KH_2PO_4 (pH 4.5) and acetonitrile (71:29, v/v), at a flow rate of 0.8 mL/min. Olaparib and an internal standard (imatinib) were well separated from the co-extracted material, with retention times of 13.6 and 11.5 min, respectively. The calibration curve for olaparib showed linearity over the concentration range of 0.10-10.0 μg/mL ($r^2 = 0.9998$). The intra- and inter-day validation coefficients ranged from 1.79 to 4.13% and 1.37 to 3.55%, respectively. Measurement accuracy ranged from − 6.07 to 3.26%, with a recovery rate of more than 91.06%. The developed method was then applied to evaluate the plasma olaparib concentrations in patients with ovarian cancer. Our findings demonstrate that HPLC-UV is an economical, simple, and sensitive method for clinical application and holds promise for the effective drug monitoring of olaparib during ovarian cancer treatment.

Keywords olaparib, PARP inhibitor, HPLC-UV, therapeutic drug monitoring, ovarian cancer

1. Introduction

Ovarian cancer has a poor prognosis, as approximately 70% of the patients with newly diagnosed advanced disease who undergo surgery and platinum-based chemotherapy relapse within the next three years (*1*). Olaparib is an oral, small-molecule inhibitor of poly(ADP)-ribose polymerase (PARP) used as maintenance therapy in patients with platinum-sensitive ovarian cancer who are being treated for relapse as well as after response to initial chemotherapy in patients with advanced ovarian cancer harboring mutations in the breast cancer susceptibility (*BRCA1/2*) genes. Olaparib is the new standard of care for ovarian cancer, leading to prolonged progression-free survival, and is administered orally as a 300 mg tablet twice daily.

In the SOLO-1 trial, which evaluated the efficacy of olaparib maintenance therapy after platinum- and taxane-based chemotherapy regimens, olaparib maintenance therapy reduced the risk of disease progression or death by 70% compared to the placebo (hazard ratio for disease progression or death, 0.30; 95% confidence interval, 0.23-0.41) (*2*). The excellent results of the SOLO-1 trial were limited to patients who could receive 300 mg olaparib twice daily for 2 years as a maintenance therapy. However, discontinuation due to adverse events was reported in 12% of patients in the SOLO-1 trial, with a dose reduction rate of 28% and a

discontinuation rate of 52%. The most common adverse events that led to discontinuation were anemia and nausea. Moreover, an exposure-toxicity relationship has been demonstrated between the probability of anemia and the minimum steady-state concentration as well as the predicted Cmax of olaparib (*3*).

A trough concentration of 2.5 μg/mL has been reported as the threshold for olaparib dose adjustment (*4*). However, it is unclear whether olaparib exposure is associated with efficacy (*5*). To date, the effective therapeutic range of olaparib has not been determined, with high interpatient variabilities of 50%, 39%, and 87% in the area under the concentration-time curve (AUC), maximum plasma concentration (Cmax), and minimum plasma concentration (Cmin), respectively, as determined using a population pharmacokinetic model (*3*). Many factors are thought to significantly affect olaparib exposure, with food shown to delay olaparib absorption, resulting in a significant decrease in Cmax, while the impact on AUC is only marginal (*6*). Furthermore, olaparib is primarily metabolized *via* cytochrome P450 (CYP) 3A, and thus, the concurrent administration of potent CYP3A inhibitors or inducers affects olaparib concentrations (*7*). High olaparib concentrations are associated with impaired renal function, necessitating the careful consideration of blood olaparib levels (*8*). Thus, the therapeutic drug monitoring (TDM) of olaparib in patients receiving the drug is essential.

To date, olaparib concentrations in the human plasma have only been determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (*9-14*). LC-MS/MS is expensive; thus, it is available only at a limited number of facilities. Meanwhile, high-performance liquid chromatography-ultraviolet (HPLC-UV) instruments remain popular, owing to their low initial cost and high utility in general hospitals. In light of this, we developed a simple HPLC-UV method to determine olaparib concentrations in patients with ovarian cancer, suitable for routine TDM in clinical practice.

2. Materials and Methods

2.1. Standards and reagents

Olaparib and imatinib were acquired from Toronto Research Chemicals Inc. (Ontario, Canada). The mobile phases comprised HPLC-grade acetonitrile, methanol, water (Kanto Chemical Co., Inc., Tokyo, Japan), and KH_2PO_4 (Wako, Osaka, Japan).

2.2. Chromatographic conditions

The HPLC system consisted of a pump (PU-4180; Jasco, Tokyo, Japan), UV detector (UV-4075; Jasco, Tokyo, Japan), and autosampler (AS-4550; Jasco, Tokyo, Japan). Analysis was performed using an octadecylsilyl column (Capcell Pak C18 MG II; 250 mm × 4.6 mm; i.d., 5 μm; Osaka Soda, Tokyo, Japan). The detection wavelength was 218 nm. The mobile phase consisted of 0.5% KH_2PO_4 (pH 4.5) and acetonitrile (71:29, v/v), which was eluted at a flow rate of 0.8 mL/min. The injection volume for HPLC analysis was 30 μL.

2.3. Calibration curve and quality control samples

Stock solutions of 1 mg/mL olaparib and imatinib were prepared in methanol. For the calibration curve, fresh blank plasma was spiked with the olaparib stock solution to obtain final concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 μg/mL. The olaparib stock solution was diluted in methanol to obtain working solutions of 0.5, 2.5, 5, 12.5, 25.0, and 50.0 μg/mL. Imatinib was diluted in methanol to obtain a working solution of 10 μg/mL. Subsequently, the prepared stock and working solutions were stored at −60°C in the dark until use.

2.4. Sample preparation

Plasma (50 μL) was pipetted into a 2.0 mL microtube. Thereafter, 10 μL of the olaparib working solution was vortexed with 50 μL of the plasma in a 2.0 mL microtube for 10 s. Subsequently, 60 μL of olaparib-spiked plasma, 10 μL of the internal standard (IS; 10 μg/mL imatinib), and 330 μL of methanol chilled to −60°C were added. The mixture was then vortexed for 1 min and centrifuged at 15,000 ×*g* for 10 min at 4°C. Finally, the resulting supernatant was transferred to an HPLC autosampler vial, and 30 μL was injected into the HPLC system.

2.5. Method validation

Method validation was based on the Guidelines for the Validation of Methods for the Quantitative Analysis of Biological Samples by the US Food and Drug Administration (FDA) (*15*). The calibration concentrations of olaparib ranged from 0.1 to 10.0 μg/mL; the recovery and accuracy of the assay were determined at these concentrations. Assay precision was evaluated by analyzing five sets of control samples on the same day (intra-day) and five different days (inter-day) at concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 μg/mL.

The stability of olaparib in plasma samples was assessed at three different concentrations (0.1, 1.0, and 10.0 μg/mL). To establish benchtop stability, five samples (n = 5) stored at 25°C for 6 h were evaluated. Processed sample stability was evaluated by storing five samples (n = 5) at 4°C for 24 h. To determine long-term stability, five samples (n = 5) were stored at −60°C for one month and three months and then assessed. Finally,

freeze-thaw stability was determined by subjecting five samples (n = 5) to three cycles of freezing at −60°C and thawing.

2.6. Clinical application

Blood samples were collected after obtaining written informed consent from patients receiving olaparib. Plasma samples were obtained by centrifuging the blood samples at 3,000× g for 5.5 min. Plasma and serum were stored at − 80°C until analysis. This study was approved by the institutional review board of Tokyo Metropolitan Bokutoh Hospital (#04–127) and conducted in accordance with the Declaration of Helsinki. Concomitant medications used in the five patients receiving olaparib were magnesium oxide, rosuvastatin calcium, loxoprofen sodium hydrate, pemafibrate, teprenone, montelukast sodium, dextromethorphan hydrobromide hydrate, ambroxol hydrobromide, tenofovir alafenamide fumarate, candesartan cilexetil, calcium sennoside A and B, anastrozole, duloxetine hydrochloride, lorazepam, metoclopramide hydrochloride, trazodone hydrochloride, zolpidem tartrate, and daikenchueto, which did not interfere with the assay.

3. Results and Discussion

In the present study, we introduce a simple and sensitive HPLC-UV method for quantifying plasma olaparib concentrations in clinical settings. Figure 1A presents the chromatogram of the blank plasma sample. Figures 1B and 1C show the representative chromatograms of plasma samples containing 0.10 μg/mL and 1.0 μg/mL of olaparib, respectively. The background of the treated blank plasma sample was clean at the olaparib and IS imatinib peak positions, with no interference from spurious peaks. Olaparib and the IS were well separated from the co-extracted materials under the chromatographic conditions, with retention times of 13.6 and 11.5 min, respectively. The six-point olaparib standard calibration curve exhibited linearity across the concentration range of 0.10-10.0 μg/mL (r^2 = 0.9998). The limits of detection and quantification for olaparib were 0.025 μg/mL and 0.10 μg/mL, respectively. The recovery rate of olaparib exceeded 91.06%. Considering this concentration range, the intra- and inter-day CVs ranged at 1.79-4.13% and 1.37-3.55%, respectively (Table 1). The assay accuracy ranged at − 6.07-3.26%.

The plasma stability of olaparib was also assessed under various conditions (Table 2). No significant olaparib degradation was observed, and final concentrations were maintained within 93.0-111.8% of the theoretical values. No interference from olaparib or the IS was observed for the endogenous substances in the blank plasma. The precision and accuracy of the intra- and inter-assay variability and stability under diverse conditions were in accordance with the guidelines outlined by the FDA (*15*). To the best of our knowledge, this is the first study to combine a liquid-liquid extraction pretreatment method with isocratic gradient elution and UV detection to achieve effective olaparib TDM.

Previously reported methods for measuring olaparib levels in human plasma samples were exclusively LC-MS/MS-based (*9-14*). However, owing to the high cost of the equipment required, it is difficult to adopt these methods in resource-limited areas. The advantage of the HPLC-UV method presented herein is that it can be

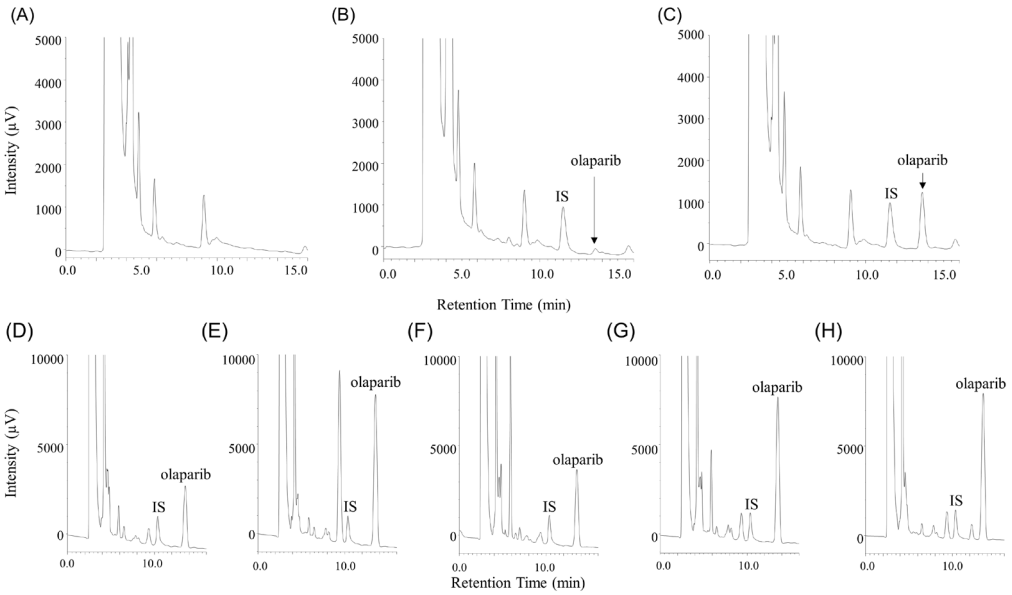

Figure 1. Chromatograms of the (A) blank plasma sample, (B) plasma sample containing 0.1 μg/mL olaparib, (C) plasma sample containing 1.0 μg/mL olaparib, and (D, E, F, G, H) those of the five patients included in this study.

Table 1. Intra-day and inter-day accuracy and precision of olaparib concentrations

Olaparib added (μg/mL)	Intra-day (n = 5)			Intra-day (n = 5)			Recovery (%)
	Mean ± SD (μg/mL)	CV (%)	Accuracy (%)	Mean ± SD (μg/mL)	CV (%)	Accuracy (%)	
0.1	0.09 ± 0.00	2.51	-6.07	0.10 ± 0.00	3.49	-1.07	100.64
0.5	0.49 ± 0.01	1.79	-2.06	0.50 ± 0.02	3.55	0.42	96.54
1.0	0.98 ± 0.03	3.36	-1.68	1.01 ± 0.01	1.37	0.71	94.54
2.5	2.53 ± 0.06	2.55	1.05	2.50 ± 0.08	2.99	0.13	94.92
5.0	5.16 ± 0.13	2.42	3.26	5.03 ± 0.10	2.05	0.51	91.06
10.0	10.13 ± 0.42	4.13	1.27	10.07 ± 0.29	2.83	0.67	92.78

CV, coefficient of variation; SD, standard deviation.

Table 2. Stability analyses under various conditions (n = 5)

Olaparib added (μg/mL)	Stability condition (%)				
	Benchtop mean ± SD	Processed sample mean ± SD	1 month mean ± SD	Long-term 3 months mean ± SD	Freeze-and-thaw mean ± SD
0.1	106.8 ± 1.2	111.8 ± 0.7	97.5 ± 1.6	97.4 ± 3.0	93.0 ± 3.3
1.0	99.6 ± 1.6	99.1 ± 1.8	100.0 ± 0.8	93.5 ± 1.3	98.1 ± 2.6
10.0	105.0 ± 0.7	100.4 ± 2.4	100.7 ± 1.3	94.5 ± 0.1	99.7 ± 0.6

SD, standard deviation.

easily transferred from one system to another. This is the most important feature when applying an existing method from the literature to the laboratory. In the past, a method for measuring olaparib levels in human plasma *via* HPLC using fluorescence detection has been reported (*16*). However, our method was the first to measure olaparib levels in human plasma using HPLC-UV. Our HPLC-UV-based method for determining olaparib plasma concentrations was established based on less-demanding techniques: liquid-liquid extraction and isocratic elution. The accessible equipment and simpler operation reduce laboratory and labor costs, making this method particularly suitable for resource-poor countries and regions.

The concomitant medications used by the five patients receiving olaparib in the current study included magnesium oxide, rosuvastatin calcium, loxoprofen sodium hydrate, pemafibrate, teprenone, montelukast sodium, dextromethorphan hydrobromide hydrate, ambroxol hydrobromide, tenofovir alafenamide fumarate, candesartan cilexetil, calcium sennoside, anastrozole, duloxetine hydrochloride, lorazepam, metoclopramide hydrochloride trazodone hydrochloride, zolpidem tartrate, and daikenchueto. No interference from these concomitant medications was noted in the chromatograms of olaparib and the IS (Figures 1D, 1E, 1F, 1G and 1H). Thus, our method achieved good olaparib extraction and avoided disturbance by plasma constituents and concomitant medications. Admittedly, this HPLC-UV method cannot achieve the same level of sensitivity as mass spectrometry-based methods. Nevertheless, the plasma olaparib concentrations of patients receiving maintenance therapy were all within the detection range of this method, highlighting its clinical applicability. It should be noted, however, that we evaluated only five patients. Therefore, we plan to validate the accuracy of our method in a larger cohort of patients.

Table 3. Plasma concentrations of olaparib in patients with ovarian cancer

Patient number	Daily dose	Plasma concentration	Timing of blood sampling after olaparib administration
1	300 mg twice daily	2.47 μg/mL	6 h
2	300 mg twice daily	6.38 μg/mL	3 h
3	200 mg twice daily	3.11 μg/mL	2 h
4	300 mg twice daily	5.98 μg/mL	3 h
5	300 mg twice daily	5.90 μg/mL	3 h

Olaparib plasma concentrations were evaluated in samples collected from five female patients diagnosed with ovarian cancer and undergoing maintenance therapy with olaparib. The mean olaparib plasma concentration was 4.77 μg/mL (range, 2.47-6.38 μg/mL) (Table 3). Olaparib reached a steady state after approximately 3-4 days and the Cmax was reached within 1-3 h after oral administration. As the Cmax had high inter-individual variability, determining the minimum concentration before a subsequent dose of the drug may be of great clinical value. We believe that monitoring trough concentrations is clinically appropriate when performing TDM of olaparib. However, this study aimed to determine whether our assay could accurately measure olaparib levels in patient samples. Therefore, we collected blood samples from patients during their outpatient visits, which allowed us to collect samples 2-6 h after olaparib administration. Patients 2, 3, 4, and 5 had samples collected 2 and 3 h after administration, and these samples were recorded as the Cmax. The mean Cmax for olaparib 300 mg

twice daily was previously reported to be 7.6 μg/mL in patients with ovarian cancer, breast cancer, and other types of solid tumors (*16*). In our study, patients 2, 4, and 5, who received olaparib 300 mg twice daily, had a lower mean Cmax, 6.09 μg/mL. One possible reason for this lower Cmax in our case was that, in previous reports (*17*), the Cmax was measured in solid tumors other than ovarian cancer. Currently, the indications of olaparib have been extended to breast, pancreatic, and prostate cancer (*18*). In the future, we hope that the assay presented herein may be used to determine the Cmax and Cmin in patients with each of these cancer types, which would facilitate the assessment of differences between tumors. In the present study, Patient 2 experienced nausea and headache, which led to the temporary discontinuation of olaparib. After resuming therapy, the Cmax determined on day 34 was 6.38 μg/mL, with an estimated trough concentration of approximately 3.0 μg/mL, greater than the threshold for dose adjustment of 2.5 μg/mL (*4*).

In conclusion, we developed a cost-effective, simple, and sensitive HPLC-UV method for measuring plasma olaparib concentrations in the clinical setting. Successful application of this assay to patient blood samples demonstrated its reliability. Further studies using this assay are required to clarify the relationship between the blood levels of olaparib and its clinical efficacy and safety.

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