Intracellular Pharmacokinetics of PARP Inhibitors in Breast and Ovarian Cancer Cells and Correlation to Drug Action: A Review

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Article info

Abstract

Recently Poly (ADP-Ribose) Polymerase inhibitor (PARPi) drugs were approved by the FDA for clinical use in breast and ovarian cancer, recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer. Olaparib an oral formulation approved for the treatment of patients with BRCA mutation and recurrent ovarian cancer; has shown to provide clinically significant benefit. Inhibition of PARP results in accumulation of single-strand breaks, leading to formation of double-strand breaks. Olaparib, a small molecule, selectively binds and inhibits Poly (ADP-Ribose) Polymerase enzyme, inhibiting its mediated repair of single-strand DNA breaks. Poly (ADP-Ribose) Polymerase inhibition enhances cytotoxicity of DNA damaging agents and reverses tumor cell chemoresistance and radio resistance. There has been limited research on the quantification of anti-cancer drugs intracellularly; very few studies have attempted to quantify Olaparib intracellularly. For the first time, intercellular quantification of PARP inhibitor was reported in two studies involving oral dosage form and the other nano-delivery system, allowing for quantification of Olaparib distribution in the nuclei, cytoplasm, liver, kidney, plasma, and urine. This review, covers articles and reports from 1992 to 2019 and is aimed at highlighting the increasing importance of intracellular quantification of anti-cancer drugs using PARP inhibitors as examples, due to limited research done on this group of drugs, only Olaparib has been reported have been determined intracellularly.

Keywords: HPLC-UV-DAD; Method validation; Intracellular drug concentration; PARP inhibitors; Olaparib

Introduction

Breast and ovarian cancer are ranked among the topmost significant types of cancer in terms of rates of incidences and mortality in 2018 by the world health organization. Together, these two cancer types are responsible for a third of mortality and prevalence worldwide. An estimation of 2.1 million diagnoses was made in 2018, 11.6% of total cancer incidence burden worldwide [1]. Unfortunately, breast and ovarian cancer incidences are still rising to date. In the recent few decades, substantial progress has been made to understand breast cancer molecular pathways, leading to development of more individualized therapies [2]. Despite this, the metastatic breast cancer 5-year survival rate remains low [3]. Breast cancer is a very heterogeneous disease, with broad differentiation and clinical behaviors [4]. Breast cancer is classified based

on immunohistochemical features: Human Epidermal Growth Factor Receptor 2 (HER2)-positive, Hormone Receptor (HR)-positive, and Triple-Negative (TN) tumors [5]. TNBCs comprise about 15 % of breast cancers overall, about 70 % in germline *BRCA1* mutation, and 20 % in *BRCA2* mutation carriers (TNBCs) characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression [6]. TNBC, compared to other types of breast cancer, has a high reoccurrence rate and a poor 5-year prognosis [7]. Targeted therapy such as endocrine therapy or Trastuzumab are unable to treat TNBCs because they lack cellular targets [8]; hence treatment of TNBC is a major challenge. In this review, we aimed at highlighting the increasing importance of intracellular quantification of anti-cancer drugs for enhancement of efficacy, reducing adverse effects, increase tolerability and new ways to maintain sensitivity and minimize

resistance using PARP inhibitors as examples. Due to limited research done on this group of drugs, only Olaparib has been determined intracellularly in the recent analysis.

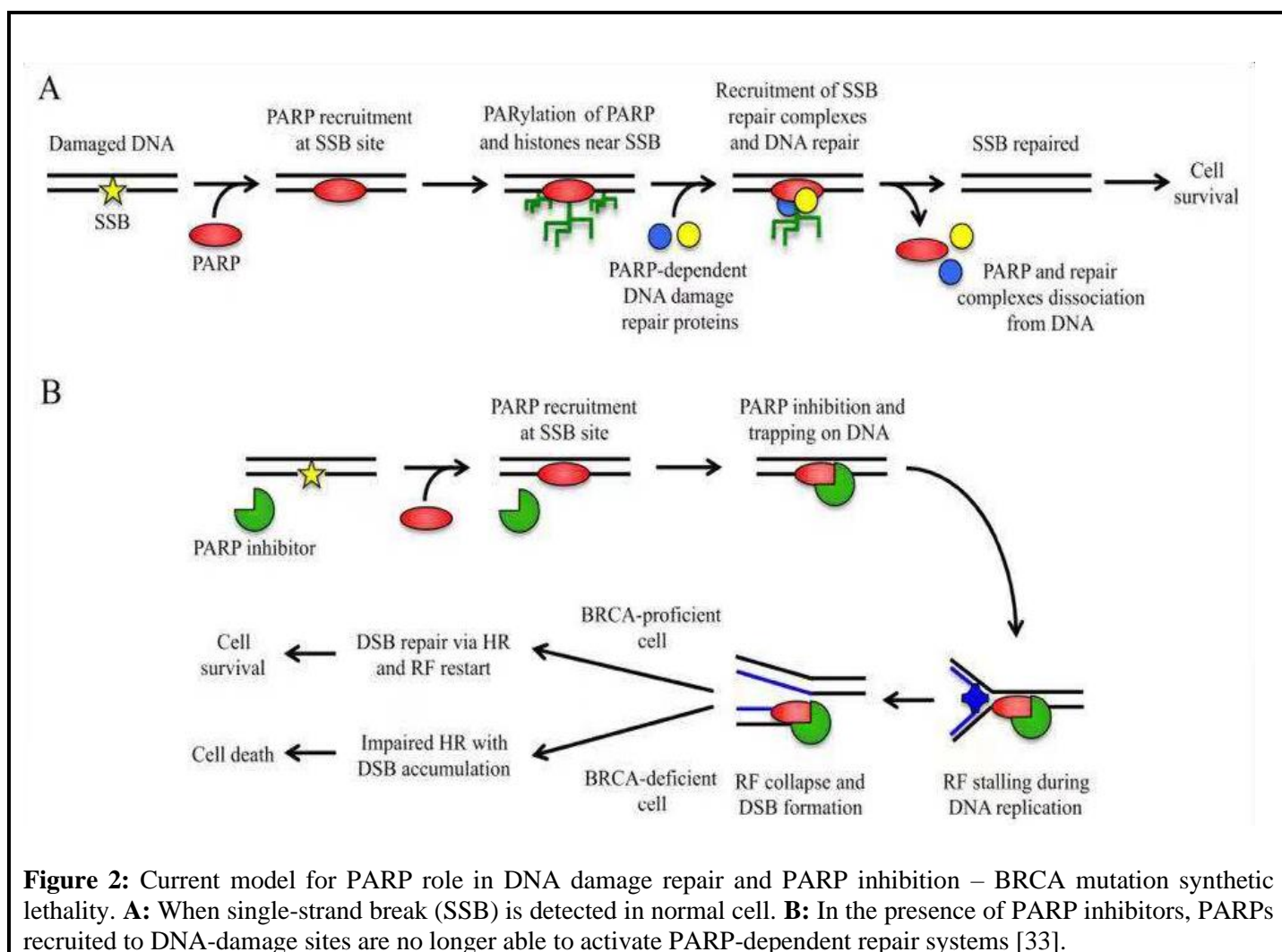**Pathophysiology, causes and molecular pathways of breast carcinogenesis**

When breast cells come into contact or interact with external factors, and due to genetic susceptibility they become cancerous [2]. Normal cells multiply and divide as many times as needed, then stop, attach to other cells and then stay in place in tissues. When cells lose the ability to divide, attach to other cells, remain in the right place and die inappropriate time, they become cancerous. The typical cell dies when they have lived their life and are no longer needed, until this time they are protected from cell death by protein clusters and pathways (figure1).
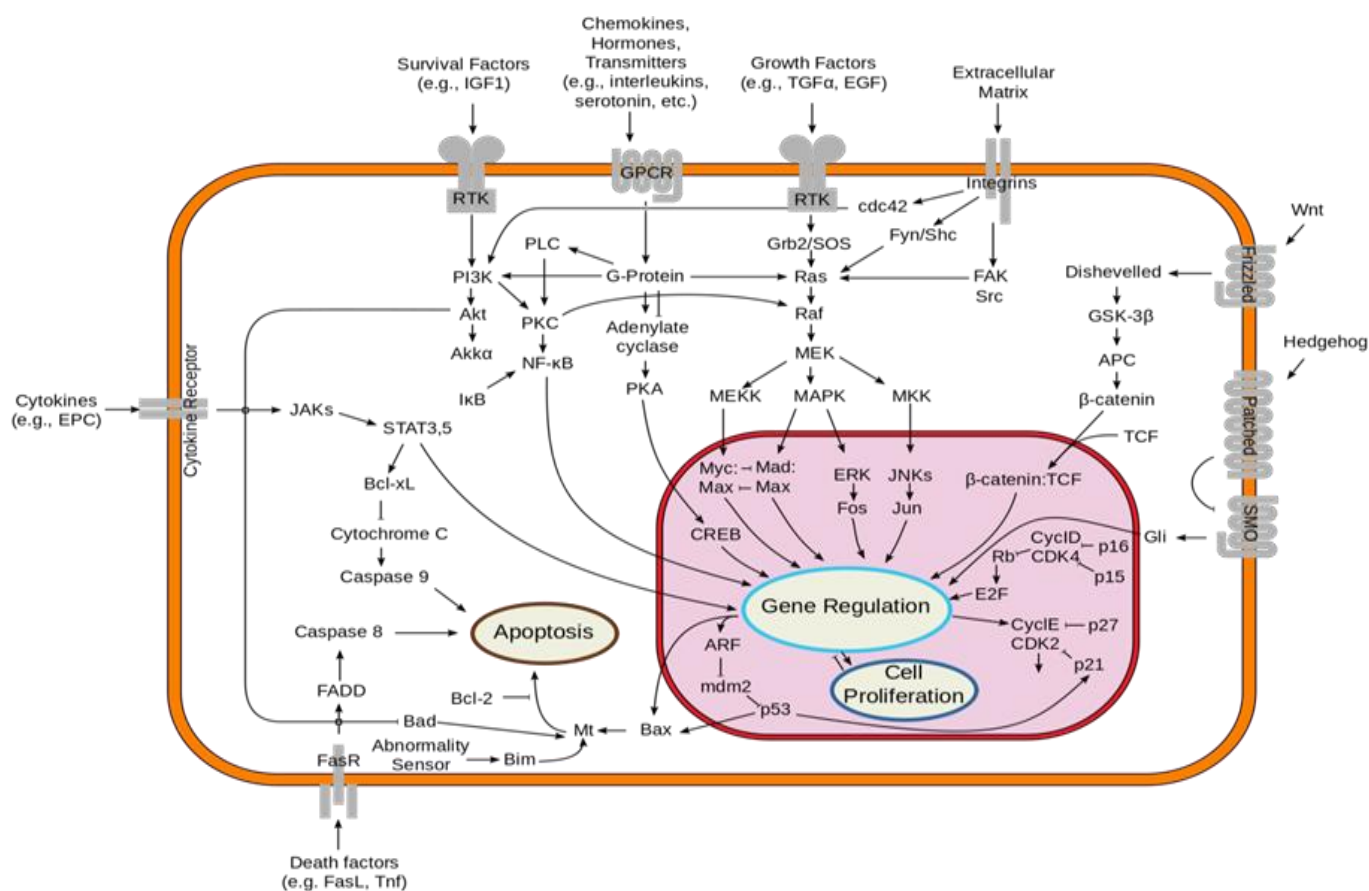
Figure 1: Signal transduction pathway.

Two protective channels are the PI3K/AKT and the RAS/MEK/ERK pathway. In specific instances, the genes on these protective pathways are mutated causing the pathway to be turned permanently "on" meaning the cell then becomes incapable of cell death at the time it is no longer needed. This step in combination with other mutations is what causes cancer. Normally, the PI3K/AKT pathway is turned off by the PTEN protein when the cell is ready for cell suicide. When the PTEN protein gene is mutated, the cancer cells avoid apoptosis because the PI3K/AKT pathway is stuck in the "on" position [9]. Estrogen exposure is experimentally identified as a link to mutations leading to breast cancer [10, 11]. In the interaction between epithelial cells and stromal cells, abnormal growth factor signaling can facilitate cancerous cell growth [12, 13]. Overexpression of leptin leads to increased cell proliferation in adipose tissue and breast cancer [14]. Hereditary breast-ovarian cancer syndrome is the familial tendency to develop these cancers. The BRCA mutations present with a 15-40 % lifeime risk od ovarian cancer and 60-85% risk of breast cancer. Mutations that occur due to drugs mechanisms to correct DNA such as p53, BRCA and BRCA2 acquired after birth or inherited, are associated wuth cancer. However, there is strong evidence between carrier families, enviromental causes and inherited mutations in BRCA1 and BRCA2 genes [15-18].

Figure 2: Current model for PARP role in DNA damage repair and PARP inhibition – BRCA mutation synthetic lethality. **A:** When single-strand break (SSB) is detected in normal cell. **B:** In the presence of PARP inhibitors, PARPs recruited to DNA-damage sites are no longer able to activate PARP-dependent repair systems [33].

DNA damage are as a result of carcinogens often requiring repairs by pathways with BRCA1 and BRCA2 [19,20]. However, only 2 to 3 % of all breast cancers are due to variations in BRCA genes [21]. Levin et al., states that half of inherited breast-ovarian cancer involve unknown genes and is not inevitable for all BRCA1 and BRCA2 mutation carriers. The protein GATA-3 controlling the expression of ER and other genes associated with epithelial distinction. The depletion of GATA-3 leads to poor prognosis and loss of differentiation due to cancer cell metastasis and invasion [22].
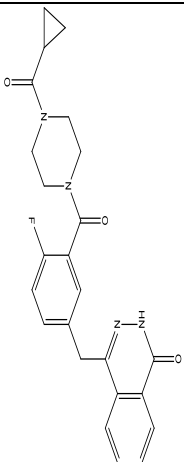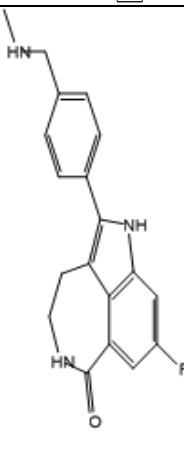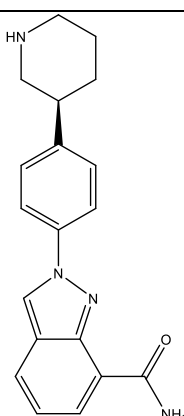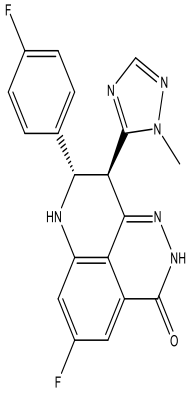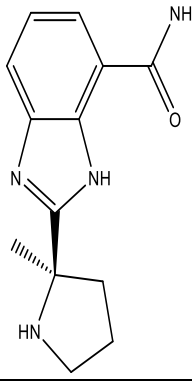
PARPs and DNA damage repair

Poly (Adenosine Diphosphate-Ribose) Polymerase (PARP) is a single strand DNA damage recognition repair protein. Accumulation of single strand breaks due to inhibition of PARP leads to accumulation of double strand DNA breaks. Cells with BRCA mutation are not able to repair DSBs hence are unable to undergo apoptosis [23] (figure 2).

PARP inhibitors

PARPi drugs recently been approved by the FDA for clinical use in breast and ovarian cancer. An oral PARP inhibitor, Olaparib has been approved for treatment of ovarian cancer and a BRCA mutation [24-27].

Table 1: List of PARP inhibitors.

Compound Company	MW (g/mol)	Structure	Ki (nM)	IC$_{50}$ (nM)	Status in clinical development	References
Olaparib (AZD2281) AstraZeneca	435.08		n/a	PARP-1:5 PARP-2:1	FDA approved for advanced ovarian cancer with gBRCAmut with ≥ 3 prior lines of chemotherapy in 2014. For recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer maintenance treatment in adult patients with incomplete or partial response to platinum-based chemotherapy in 2017.	[34,35]
Rucaparib (CO-338) Clovis	421.36		PARP-1:1.4 PARP-2: n/a	PARP-1:0.8 PARP-2:0.5	FDA approved for advanced ovarian cancer with gBRCAmut or sBRCAmut with ≥ 2 lines of chemotherapy in 2016. For recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer maintenance treatment in adult patients with incomplete or partial response to platinum-based chemotherapy in 2018.	[36,37]
Niraparib (MK-4827) Tesaro	320.39		n/a	PARP-1:3.8 PARP-2:2.1	FDA approved for the recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who have a complete or partial response to platinum chemotherapy	[38]

Talazoparib (BMN-673) Pfizer	380.35		PARP-1:1.2 PARP-2:0.85	PARP-1:0.57 PARP-2: n/a	In Phase III study as monotherapy in patients with locally advanced or metastatic breast cancer with gBRCAmut in 2017	[39]
Veliparib (ABT-888)	244.29		PARP-1:5.2 PARP-2:2.9	n/a	In Phase III studies as combination therapy with chemotherapy in patients with breast and ovarian cancer with gBRCAmut or sBRCAmut and lung cancer.	[40]

MW: Molecular weight; gBRCAmut: Germline BRCA mutations; sBRCAmut: Somatic BRCA mutations; FDA: United States Food and Drug Administration; N/A: not available.

The OlympiAD trial designed to compare the safety and efficacy of Olaparib with the physician's choice of standard therapy, single-agent chemotherapy, among patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Phase. Olaparib showed clinical efficacy in both breast and ovarian cancer in phase 1 [28]. Talazoparib at the recommended dose of 1.0 mg/day demonstrated single agent antitumor activity and was well tolerated in phase 2. Talazoparib also demonstrated encouraging activity in patients with gBRCA1/2mMBC, who previously received platinum-based therapy or various chemotherapeutic regimens [28-32].
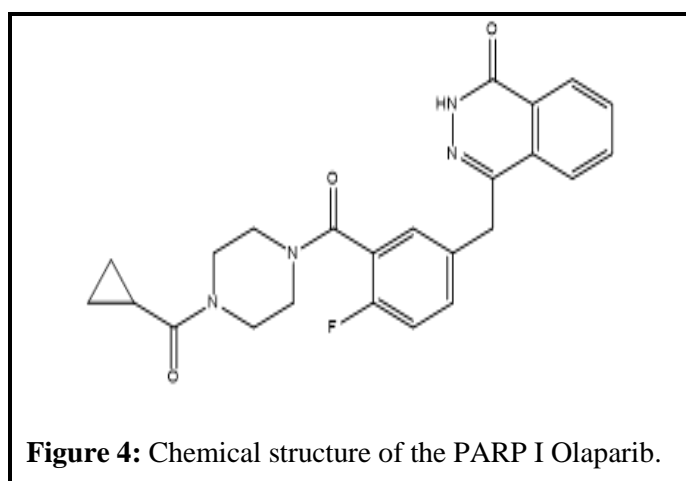
Clinical pharmacokinetics of PARP inhibitors

The clinical pharmacokinetics of PARP inhibitors have been summarized in table 2. Olaparib [41], Rucaparib [42], Niraparib [43], Talazoparib [44] and Veraparib [45].

Methodological considerations

Olaparib (figure 4), Veliparib, Niraparib and Rucaparib; PARP inhibitors in combination or as standalone therapy have recently reached advanced clinical trials in ovarian and breast cancers. The first to gain regulatory approval for treatment of BRCA-mutated ovarian cancer by European Commission in 2014 and FDA in 2015 was Olaparib [46-48]. The sensitivity of tumor cells to PARP inhibitors has been shown to vary widely in preclinical and clinical studies hence treatment efficacy needs to be optimized. The amount of PARP inhibitors that reaches cell interior compartments, influences sensitivity and effectiveness of therapy as PARP is an intracellular target [49].

Figure 4: Chemical structure of the PARP I Olaparib.

Table 2: Summary of pharmacokinetic parameters of PARP inhibitors.

Drug	T_{max} (h)	$T_{1/2}$ (h)	AUC (µg.h/mL)	C_{max} (µg/mL)	CL/F (L/h)	Vz/F	References
Olaparib capsule formulation 300 mg	1.49 (0.57-3.05)	13.02 (8.23)	55.20 (67.4)	8.05 (24.3)	6.36 (3.47)	112.1 (59.84)	[71]
Olaparib tablet formulation 300 mg single dose (fasted)	1.50 (0.50–5.85)	12.2 (5.31)	43.6 (54.3) [AUC_t] 43.0 (55.2) [AUC_∞]	7.00 (35.0)	7.95 (4.23)	146 (142)	[72]
Olaparib tablet formulation 300 mg single dose (fed)	4.00 (1.00–12.0)	12.2 (5.31)	46.0 (56.6) [AUC_t] 45.4 (57.1) [AUC_∞]	5.48 (40.5)	7.55 (3.99)	127 (107)	[72]
Veliparib monotherapy 40 mg (4 x 10 mg, fasting)	1.2 ± 0.8	5.9 ± 1.3	2.23 ± 0.82 [AUC_t] 2.43 ± 1.07 [AUC_∞]	0.36 ± 0.13	19.0 ± 7.36	NA	[73]
Veliparib monotherapy 40 mg (4 x 10 mg, fed)	1.2 ± 0.7	5.8 ± 1.2	2.45 ± 0.93 [AUC_t] 2.65 ± 1.17 [AUC_∞]	0.37 ± 0.12	17.3 ± 6.41	NA	[73]
Veliparib monotherapy 40 mg (1 x 40 mg, fasting)	1.3 ± 0.9	5.8 ± 1.3	2.24 ± 0.98 [AUC_t] 2.45 ± 1.24 [AUC_∞]	0.34 ± 0.12	19.5 ± 7.66	NA	[73]
Veliparib monotherapy 40 mg (1 x 40 mg, fed)	2.5 ± 1.1	5.8 ± 1.4	2.14 ± 0.80 [AUC_t] 2.35 ± 1.06 [AUC_∞]	0.28 ± 0.09	19.7 ± 7.51	NA	[73]
Veliparib metabolite M8	2.4 (3.5–9.8)	–	0.3–1.9 [AUC_{int}]	0.011 (0.007-0.014)	NA	NA	[74]
Niraparib 300 mg/day	3.1 (2.0–6.1)	a	14.117 (AUC_{24})[b]	1.921[b]	NA	NA	[75]
Niraparib metabolite: unlabeled M1 plasma	9.02	78.4	41.2(AUC_∞)	476	NA	NA	[76]

PARP: Poly (ADP ribose) polymerase; T_{max}: Time to reach C_{max}; $t_{1/2}$: Terminal elimination half-life; AUC: Area under the plasma concentration-time curve; C_{max}: Peak concentration; CL/F: Apparent clearance; Vz/F: Apparent volume of distribution; AUC_t: AUC from time zero to time t; AUC_∞: AUC from time zero to infinity; NA: Not available; a:Intensive pharmacokinetic sampling was not performed (no drug holiday between the first and second courses before the third protocol amendment, and thus half-life could not be calculated for the first two doses); AUC^b: 0–12 h and concentration after 12 h reported because sampling ended at 12 h on day 1 of the second course.

Intracellular drug disposition is determined by drug metabolizing enzymes and transporting proteins. This was discovered during an early preclinical trial to be important as an essential resistance mechanism for PARPi [50-53]. Intercellular quantification methods for PARP inhibitors in tumor cells offer the advantage of dose optimization and improved predictions of drug response. To evaluate the relationship between PARP

inhibitors intracellular concentration and treatment efficacy, reliable quantification methods are warranted. Previously intercellular quantification of drugs was challenging and radiolabeled analog (are not readily available) were relied on to assist in quantification [54, 55]. Therefore, LC-MS/MS is a susceptible and specific technique for low drug level detection of drugs in cells, but sample preparations are time consuming and instruments are expensive [56].

LC-MS/MS methods for quantification of PARPi in human plasma, cell culture medium, brain and tumor cells were reported for the first time [57, 58]. Oplustilova et al. reported intracellular concentration of an Olaparib analog by LC-MS/MS but offered no details on analytical methods used [62]. Flow cytometric analysis was used for single-cell analysis and assessment of intracellular concentration of fluorescent drugs [59, 63-65]. This technique is in the early development stage, and few drugs have intrinsic fluorescence. With available equipment, HPLC with UV-DAD analysis both allow for sensitive quantitation of numerous compounds [66].

Once a drug enters a cell often facilitated by transporters, it accumulates in organelles. When in the cytoplasm, essential molecules accumulate in cell organelles. To predict a drug's pharmacologic impact accurately, information about its level at the target site is essential but is difficult to determine. Techniques combining mass spectrometry and imaging techniques such as matrix-assisted laser desorption/ionization, secondary ion mass spectrometry (SIMS), and Nano SIMS offer promise in combating this problem [49]. The main limitation in intercellular drug quantification

is lack of suitable methods and techniques to determine drug distribution within cells [67]. New methods of intercellular drug quantification are on the horizon in hopes to complement advances in time-lapse confocal microscopy used to study mannerisms of intracellular organelles in living and intact animal cells [67]. For a drug to elicit its pharmacological action it must reach the desired target site with effective and substantial concentration, in anticancer therapy many of these targets are intracellular. The study and research of the distribution of drugs into and within the cell are most neglected research areas in pharmacokinetics, pharmacology and therapeutics [69]. The use of drug concentration in plasma, rather than dose, in pharmacology, therapeutics and safety assessment although being a critical step forward, urgently needs to be replaced by information about intracellular concentrations and distribution. This review aims emphasize the increasing need for determination of drug concentrations and outcomes within the cell and its organelles. This topic has become of increasing importance in anticancer drug discovery as cancer drugs have intercellular drug targets such as mitochondria, oligonucleotides, antibodies and lysosomes [68].

Mechanisms influencing intracellular accumulations

Intracellular drug accumulation is influenced by drug transporting proteins and enzymes whose expressions are cell and tissue dependent. Intracellular drug disposition is determined by drug metabolizing enzymes and transporting proteins [79, 80]. Cell interior drug concentration is increased by uptake transporters and reduced by efflux enzymes and transporters [80-83] (figure 5).
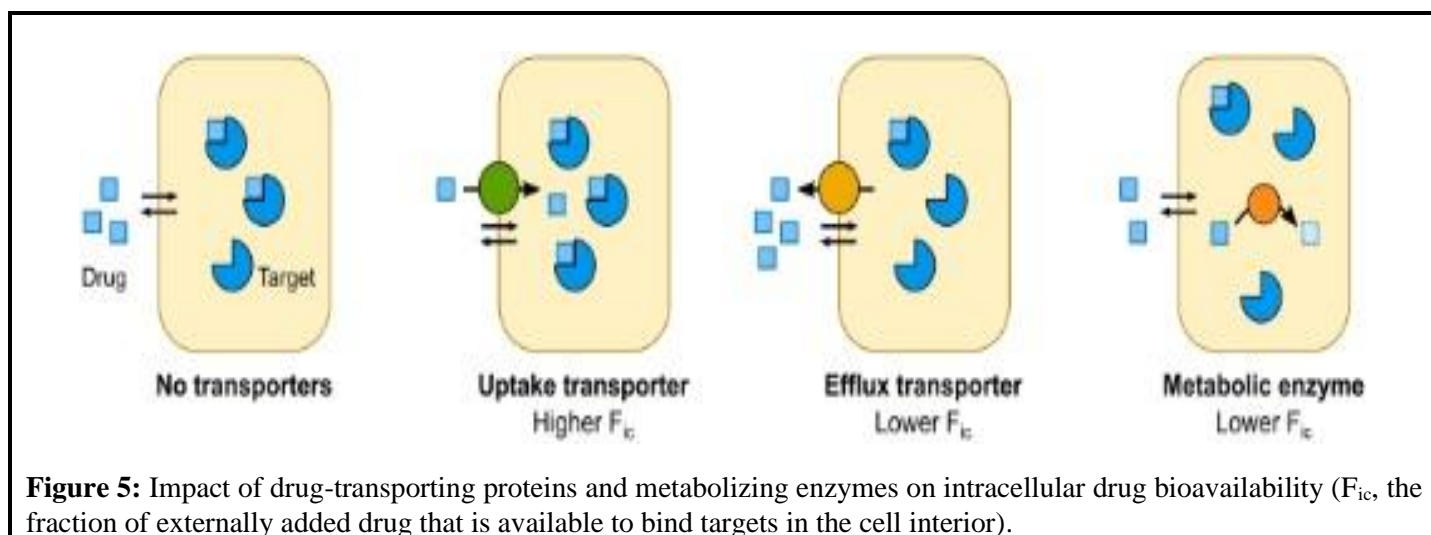
Figure 5: Impact of drug-transporting proteins and metabolizing enzymes on intracellular drug bioavailability (F_{ic}, the fraction of externally added drug that is available to bind targets in the cell interior).

Clinical studies of intracellular concentrations

This review was to highlight the increasing importance of intracellular quantification of anti-cancer drugs using PARP inhibitors as examples, due to limited research having been done on this group of drugs only Olaparib is reported to have been quantified intracellularly. For the first time, Olaparib was quantified inside cancer cells by use of HPLC-UV-DAD method capable of quantifying Olaparib in the whole cell in the range 200 - 2000 ng/mL [66]. The method allowed for quantification of drug concentration in cancer cells allowing for dose optimization and enhanced treatment response estimations [66]. In another recent study, a method was developed to quantify Olaparib encapsulated in ferritin-nano-carriers in nano drug development and applied to human BRCA-mutated cell model to quantify the Olaparib distribution. The method allowed, the quantification of low levels of Olaparib in different biological complex matrices for the first time; such as cell compartments, mouse tissues, plasma and urines, and precisely, the requirement of low volume of plasma and urine, rendered the particular method attractive for pharmacological research. In this method Olaparib was quantified within the range of 0.10 – 10 ng/mL in cells cytoplasm and culture medium, 0.50 - 10 ng/mL in nuclei, 0.50 – 100 ng/mL in urine and plasma and 10 – 500 ng/mL in liver and kidney [77].
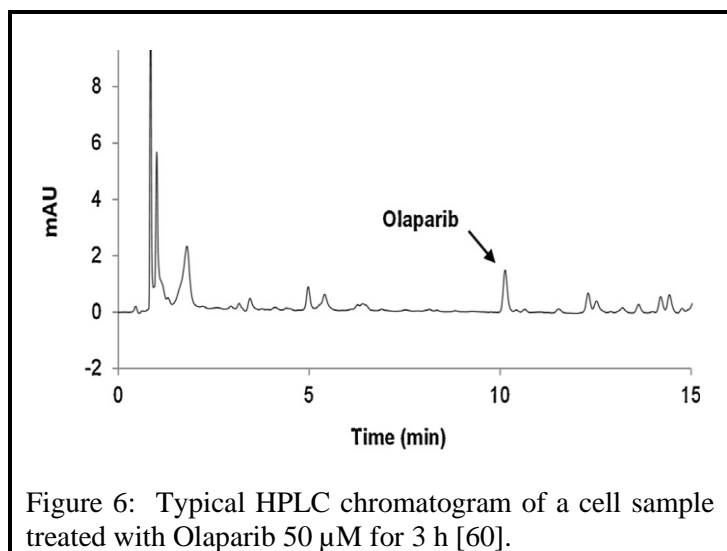

Figure 6: Typical HPLC chromatogram of a cell sample treated with Olaparib 50 μM for 3 h [60].

Method applicability

The described method by Daumar et al. was developed and validated for intracellular determination of Olaparib in cultured cells. A suitable and sensitive method with limited sample preparation steps [64].

A reversed-phase HPLC with UV detection method, with readily available equipment, simple and rapid and inexpensive technique than tandem mass spectrometry and can be extended to other PARP inhibitors [60]. The method by daurmar compared with that published by Roth et al. [55], which is to date the highest sensitive and robust approach developed, in terms of recovery of analyte, range of calibration and sensitivity. Olaparib quantified over the calibration range of 200 - 2000 ng/mL with limit of quantification of 0.10 ng/mL intracellularly. Addition of urine, kidney and liver tissues makes this method flexible and broadly applicable to clinical and pharmacological studies of Olaparib elimination. Finally, a method based on nano-delivery system was also reported for the first time [71,78].

Future Prospective

The aim was to highlight the increasing importance of intracellular quantification for dose characterization, verification of drug accumulation at target site and mechanism of action of anti-cancer drugs using PARP inhibitors as examples, due to limited research done on this group of drugs only Olaparib has been reported and determined intracellularly. To Pharmacologists, pharmacokineticists and clinical pharmacologist, considerable attention needs to be drawn towards drug concentrations within the cell and other essential organelles within. The time has come to rethink the use of plasma concentration and volume at steady state as a critical way of explaining concentration in the extracellular fluid space/ drug distribution. This knowledge will go a long way in enhancing efficacy, reducing adverse effects, tolerability and new ways to maintain sensitivity and minimize resistance to PARP inhibitors. Knowledge and understanding of drug entry into specific tissues, cells and active site are becoming substantially crucial as a significant component of therapeutics, pharmacologic and clinical research.

Conflict of Interest

The authors declare no potential conflict of interest.

Acknowledgement

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