Radiotherapy and Oncology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original article

Improved pharmacodynamic (PD) assessment of low dose PARP inhibitor PD activity for radiotherapy and chemotherapy combination trials

Rosemarie de Haan [a,b,1], Dick Pluim [c,1], Baukelien van Triest [a], Michel van den Heuvel [d], Heike Peulen [a], Damien van Berlo [b], Jay George [e], Marcel Verheij [a,b], Jan H.M. Schellens [c,f,g], Conchita Vens [a,b,*]

[a] Department of Radiation Oncology; [b] Division of Biological Stress Response; [c] Division of Molecular Pathology; [d] Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands; [e] Trevigen, Inc., Gaithersburg, MD, USA; [f] Department of Clinical Pharmacology, The Netherlands Cancer Institute, Amsterdam; and [g] Department of Pharmaceutical Sciences, Utrecht University, The Netherlands

ARTICLE INFO

Article history:
Received 26 June 2017
Received in revised form 11 October 2017
Accepted 13 October 2017
Available online xxxx

Keywords:
Pharmacodynamic assay
PARP inhibitor
Chemoradiation
Olaparib (Lynparza™)
RT combination trials

ABSTRACT

Background: PARP inhibitors are currently evaluated in combination with radiotherapy and/or chemotherapy. As sensitizers, PARP inhibitors are active at very low concentrations therefore requiring highly sensitive pharmacodynamic (PD) assays. Current clinical PD-assays partly fail to provide such sensitivities. The aim of our study was to enable sensitive PD evaluation of PARP inhibitors for clinical sensitizer development.

Material and methods: PBMCs of healthy individuals and of olaparib and radiotherapy treated lung cancer patients were collected for ELISA-based PD-assays.

Results: PAR-signal amplification by *ex vivo* irradiation enabled an extended quantification range for PARP inhibitory activities after *ex vivo* treatment with inhibitors. This "radiation-enhanced-PAR" (REP) assay provided accurate IC50 values thereby also revealing differences among healthy individuals. Implemented in clinical radiotherapy combination Phase I trials, the REP-assay showed sensitive detection of PARP inhibition in patients treated with olaparib and establishes strong PARP inhibitory activities at low daily doses.

Conclusions: Combination trials of radiotherapy and novel targeted agent(s) often require different and more sensitive PD assessments than in the monotherapy setting. This study shows the benefit and relevance of sensitive and adapted PD-assays for such combination purposes and provides proof of clinically relevant cellular PARP inhibitory activities at low daily olaparib doses.

Pharmacodynamic (PD) assays that measure the effect(s) of a drug on its specific biological target(s) are essential for clinical drug development. PD data support clinical decision making concerning the optimal dose and duration of a drug therapy and, in case of combination treatment strategies, the optimal dosing and sequence of the different components [1,2]. Furthermore, it may allow individualized treatment selection or adaptation if drug responses vary among patients.

Next to established monotherapy indications in certain cancers, PARP (poly(ADP-ribose) polymerase) inhibitors are promising anticancer drugs to be combined with chemotherapy and/or radiotherapy. A range of different PARP inhibitors is currently under clinical evaluation, of which olaparib (Lynparza™) is the first FDA and EMA approved drug. PARP is an enzyme involved in the repair of DNA lesions such as single-strand breaks. Upon its activation, PARP produces poly(ADP-ribose)-polymers (PAR) at the expense of nicotinamide adenine dinucleotide (NAD). PARP inhibition by pharmaceutical drugs decreases PAR levels in cells. Several PD assays that measure PARP activity in tumour cells and in peripheral blood mononuclear cells (PBMCs) have been used in clinical studies [3–12].

Currently, there is only one clinically validated PD assay that quantifies basal PAR levels (generated by PARP through endogenously induced DNA damage), both in PBMCs and tumour cells, using an ELISA based method [6–8]. Through extensive NCI (US National Cancer Institute) studies optimizing its clinical application, this assay was able to support the clinical development of PARP inhibitors [13–17]. Especially in PBMCs, however, basal PAR levels can be low with a high day-to-day variation within the same individual [6,7], making sensitive quantification of PAR levels upon

* Corresponding author at: Division of Biological Stress Response and Department of Radiation Oncology, Netherlands Cancer Institute, Plesmanlaan 122, 1066 CX Amsterdam, The Netherlands.
E-mail address: c.vens@nki.nl (C. Vens).
[1] Both authors contributed equally (shared first authorship).

https://doi.org/10.1016/j.radonc.2017.10.017
0167-8140/

PARP inhibition difficult. Applicability of this PD assay is therefore limited to patients with sufficiently high PAR levels and it can be difficult to detect clinically relevant reductions in PAR levels. This is particularly important in the development as sensitizer in combination treatments, as PARP inhibitors have been shown to be effective chemo- and radiosensitizers at much lower concentrations and shorter duration [18,19]. Thus, sensitive and robust PD assessments are needed in such combination trials.

The aim of our study was to evaluate PARP inhibition at low drug dose levels for novel combination trials and therefore to achieve highly sensitive quantification of PAR levels and PAR reduction due to PARP inhibition by a clinically applicable PD assay.

Material and methods

Healthy individual and clinical trial patient material

Healthy individual (HI) blood was drawn in the morning after 1 h fasting; informed consent was obtained. Clinical PD data are from non-small cell lung cancer (NSCLC) patients included in a clinical Phase I trial (NCT01562210). Patients were treated with a radical standard of care chemoradiotherapy schedule (66 Gy with daily cisplatin at 6 mg/m^2) combined with olaparib 25 mg bidaily (tablet formulation). The study was performed according to ICH-GCP guidelines after approval by the hospital's ethics committee and national regulatory body. All patients gave written, informed consent prior to undergoing study-related procedures. Patient blood samples were collected prior and during treatment at a steady-state minimal concentration (C-min) level of olaparib and after completion.

Radiation and reagents

PBMCs were exposed to radiation using the Gammacell®40-Exactor (Best Theratronics Ltd., Ottawa, Canada). Olaparib for the *ex vivo* analyses in HI was purchased from Sequoia Research Products (Pangbourne, UK). Niraparib was kindly provided by the Slotervaart-Pharmacy (Amsterdam, The Netherlands). Stock-solutions were prepared in dimethylsulphoxide at a concentration of 5 mM (olaparib) and 0.31 mM (niraparib).

PBMC lysate preparation

Cell lysates were prepared following the NCI advised protocol for clinical use [8] with some minor adaptations as listed in Sup. Table S1. Samples prepared in the 'REP-assay' (Radiation Enhanced Assay) were irradiated with 8 Gy on ice and incubated for 1 h on ice. Intact PBMCs in plasma of healthy (and untreated) individuals were incubated *ex vivo* with olaparib and niraparib at 37° C 1 h before irradiation. Olaparib plasma levels and protein-binding after *ex vivo* incubation were comparable to the clinical situation, as determined by HPLC-MS/MS (Sup. Fig. S1).

PAR assay

Cellular PAR levels were measured by using the HT-PARP *in vivo* Pharmacodynamic Assay II, following the NCI protocol [8] using a Tecan-Infinite-200-Pro. Plates and kits were provided by Trevigen®.

Data analysis and statistics

PAR levels (presented in pg/1E7 PBMCs) were calculated from the linear fits of the PAR standard curves. All further quality control steps and criteria were followed as recommended in the NCI protocol [8] and are specified in Sup. Table 1 together with lower limit of quantification (LLOQ) definition and IC50 (half maximal inhibitory concentration) and E-max (maximal effect) calculations [20]. One-way ANOVA tests and Tukey's multiple comparison test were used and differences were considered significant if P-value < 0.05.

Results

Basal PAR levels in PBMCs

PAR levels in peripheral blood mononuclear cells (PBMCs) of 10 healthy individuals (HIs, Sup. Table S2) were quantified following the clinically validated NCI developed method [8], herein termed NCI-protocol. Consistent with literature [6,16], using this NCI-protocol (i.e. without an *ex vivo* irradiation step) we found a wide range of PAR levels among different HIs: 52–145 pg/1E7 cells with a median of 107 pg/1E7. Basal PAR levels were quantifiable in all. It has been suggested that a minimum of 90% inhibition is required for efficient monotherapy activity [3]. Assuming efficient inhibition, a simulated 90% reduction of PAR levels would only be quantifiable in one out of ten HIs. Our data suggest that basal PAR levels in PBMCs are often too low and too close to background levels to quantify PAR reduction accurately.

PAR amplification after ex vivo irradiation

To amplify the low basal PAR signal, which is based on low levels of endogenous DNA damage, we induced DNA damage by *ex vivo* irradiation. PAR levels increased after irradiation and rapidly decreased with increasing incubation times (Fig. 1A). After incubation on ice, however, PAR level values increased and stabilized after 1 h (Fig. 1B). Radiation increased PAR levels in a dose-dependent and linear manner up to 32 Gy (Fig. 1C). *Ex vivo* irradiation of PBMCs did not negatively influence the stability of PAR levels in cell lysates (Sup. Fig. S2). For subsequent analyses with this assay, termed REP (Radiation-Enhanced-PAR) assay, we chose a radiation dose of 8 Gy followed by an 1 h incubation on ice that provides sufficient PAR signal amplification within a reasonable radiation time-frame. We conclude that this strongly amplifies PAR signal in a strictly linear manner in PBMCs and could therefore enable the sensitive quantification of changes in PAR levels by inhibitors.

Development of the REP-assay

We next compared PAR levels induced by *ex vivo* radiation in a panel of HIs and in different blood samples of the same HI. We found a strong induction of PAR levels in all HIs (Fig. 2A and B). PAR induction rates varied among HIs (range 114–308 pg PAR/1E7 cells per Gy, Sup. Fig. S3). Nevertheless, the day-to-day variation in PAR levels was smaller in all but one HI in the REP-assay than when following the NCI-protocol (Sup. Fig. S4). PAR reduction of 90% would be quantifiable in all HI and of 99% in some HI (Fig. 2C). From this we predict that *ex vivo* irradiation enables accurate quantification of PAR levels in PBMCs even upon efficient PARP inhibition in almost all individuals.

PARP inhibition dynamics after ex vivo irradiation

To explore this further, we incubated intact PBMCs from HIs *ex vivo* with the PARP inhibitor olaparib at different concentrations. Olaparib inhibits radiation-induced PAR formation at all tested radiation doses (Fig. 3A). The relative inhibition values are similar in non-irradiated and irradiated PBMCs. There is also no apparent difference in the inhibition values at different radiation doses (Fig. 3B). Hence, there is no apparent interaction between radiation dose and PARP inhibitor activity.

Please cite this article in press as: de Haan R et al. Improved pharmacodynamic (PD) assessment of low dose PARP inhibitor PD activity for radiotherapy and chemotherapy combination trials. Radiother Oncol (2017), https://doi.org/10.1016/j.radonc.2017.10.017