Inhibition of PARP-1 by Olaparib (AZD2281) Increases the Radiosensitivity of a Lung Tumor Xenograft

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Abstract

PARP-1 is a critical enzyme in the repair of DNA strand breaks. Inhibition of PARP-1 increases the effectiveness of radiation in killing tumor cells. However, although the mechanism(s) are well understood for these radiosensitizing effects in vitro, the underlying mechanism(s) in vivo are less clear. Nicotinamide, a drug structurally related to the first generation PARP-1 inhibitor, 3-aminobenzamide, reduces tumor hypoxia by preventing transient cessations in tumor blood flow, thus improving tumor oxygenation and sensitivity to radiotherapy. Here, we investigate whether olaparib, a potent PARP-1 inhibitor, enhances radiotherapy, not only by inhibiting DNA repair but also by changing tumor vascular hemodynamics in non–small cell lung carcinoma (NSCLC). In irradiated Calu-6 and A549 cells, olaparib enhanced the cytotoxic effects of radiation (sensitizer enhancement ratio at 10% survival = 1.5 and 1.3) and DNA double-strand breaks persisted for at least 24 hours after treatment. Combination treatment of Calu-6 xenografts with olaparib and fractionated radiotherapy caused significant tumor regression (P = 0.007) relative to radiotherapy alone. To determine whether this radiosensitization was solely due to effects on DNA repair, we used a dorsal window chamber model to establish the drug/radiation effects on vessel dynamics. Olaparib alone, when given as single or multiple daily doses, or in combination with fractionated radiotherapy, increased the perfusion of tumor blood vessels. Furthermore, an ex vivo assay in phenylephrine preconstricted arteries confirmed olaparib to have higher vasodilatory properties than nicotinamide. This study suggests that olaparib warrants consideration for further development in combination with radiotherapy in clinical oncology settings such as NSCLC.

Introduction

The induction and repair of DNA double-strand breaks (DSB) are a major determinant of cellular radiation sensitivity. Manipulation of the repair of these or precursor DNA lesions may therefore influence the efficacy of radiotherapy treatment in cancer.

PARP-1 is a 116-kDa nuclear protein that efficiently detects DNA single-strand breaks (SSB). The enzyme cleaves NAD into branched polymers of ADP-ribose, which are transferred to a set of nuclear proteins, causing chromatin relaxation and recruitment of other repair proteins into the damaged site (1). This property is essential for the surveillance and maintenance of genome integrity, and PARP-1 has subsequently been referred to as the Cinderella of the genome (2).

The study of PARP-1 as a potential molecular target in cancer therapy commenced in the 1980s with the development of 3-substituted benzamides as inhibitors (3–5). Inhibition of PARP-1 by these compounds compromised DNA repair in vitro and resulted in hypersensitivity of cells to treatment with radiation or monofunctional alkylating agents (6–10). Subsequently, cells with genetic deletion of PARP-1 were shown to be more sensitive to ionizing radiation than cells with functional PARP-1 (11). Evidence that inhibition of PARP-1 activity by genetic and pharmacologic methods enhanced the effects of DNA-damaging agents such as radiation, stimulated interest in generating novel inhibitors with increased potency, suitable pharmacokinetics, and reduced toxicity (12). A number of clinical candidates have since emerged and these include olaparib (AstraZeneca), ABT-888 (Abbott Laboratories), iniparib (BiPar Sciences/Sanoﬁ-aventis), AG014699 (Pfizer Inc.), INO-1001 (Inotek/Genentech), MK-4827 (Merck), CEP-8933/CEP-9722 (Cephalon), and GPI 21016 (MGI Pharma; refs. 13, 14). Studies have shown that these novel PARP inhibitors potentiate the cytotoxic effects of radiation both...
in vitro and in vivo (15–19); for example, ABT-888, AG014699, and AG14361 potentiated radiation therapy in lung and colorectal cancer xenografts (17, 19, 20). Furthermore, in the studies reported by Calabrese and colleagues (19) and Ali and colleagues (21), the PARP inhibitors AG14361 and AG014699 were shown to potentiate the effects of both radiation and chemotherapy. It was shown that these compounds not only affected DNA repair but also modified the tumor vasculature. This latter effect was similar to that seen previously for the agent nicotinamide, which is structurally related to the current PARP-1 inhibitors (22, 23). Therefore, it was hypothesized that the increased antitumor effects seen with AG14361 and AG014699 could also involve increased tumor oxygenation and improved drug delivery.

Olaparib, also known as AZD2281 or KU-0059436 (developed by KuDOS Pharmaceuticals, and later AstraZeneca), is a potent inhibitor of both PARP-1 and PARP-2. This agent has been used successfully in the context of synthetic lethality in the treatment of tumors with BRCA mutations, as well as used in combination with platinum-based drugs (24–26). It is currently at the end of phase II clinical trials after successful phase I studies where it was used as a single agent in cancer patients with BRCA1 and BRCA2 mutations (27–29). Radiosensitizing properties of olaparib have been previously described in glioblastoma multiforme cell lines and in cells deficient in DNA DSB repair (18, 30). However, the potential of olaparib to act as a radiation sensitizer for the treatment of tumors in vivo has not yet been established.

In this study, we report the use of olaparib when combined with radiotherapy to treat non–small cell lung carcinoma (NSCLC) cells in vitro and when grown as xenografts in nude mice. We show that olaparib increases the radiation sensitivity of NSCLC cells following a single dose of radiation in vitro and in a fractionated radiation treatment schedule in vivo. In addition, the ability of olaparib to modify tumor vasculature when used alone or in combination with fractionated radiotherapy is shown. Therefore, the underlying mechanisms for the antitumor effects of olaparib when combined with radiation in vivo are likely to be due to both compromising DNA repair and increasing tumor vessel perfusion.

Materials and Methods

Cell culture

Human NSCLC Calu-6 and A549 cell lines were obtained from and authenticated by American Type Culture Collection. Upon receipt, cells were banked, and passaged for less than 6 months before use in this study. Both cell lines were cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum and 2 mmol/L L-glutamine, and incubated in standard culture conditions (95% air and 5% CO2 at 37°C). Cells were routinely screened for Mycoplasma using PlasmoTest (Source BioScience Autogen). All cell culture reagents were purchased from Invitrogen Gibco unless otherwise stated.

Olaparib and nicotinamide formulation

Olaparib was supplied by AstraZeneca and nicotinamide was purchased from Sigma. For in vivo studies, olaparib was administered at a dose of 50 mg/kg orally in vehicle (PBS containing 10% dimethyl sulfoxide (DMSO) and 10% 2-hydroxy-propyl-β-cyclodextrin (Sigma), and nicotinamide was given intraperitoneally at a dose of 1 g/kg in sterile 0.9% saline solution.

Clonogenic survival assays

Cells in exponential phase were plated at low densities (1 × 102 to 3 × 104 cells) and allowed to attach overnight in standard culture conditions. Cells were exposed to olaparib 2 hours before irradiation with 2, 4, and 6 Gy using 250 kVp X-rays delivered at 12 mA (dose rate of 0.795 Gy/min). The plates were returned to standard culture conditions for additional 22 hours and continuous exposure to olaparib. The plates were incubated for 8 to 10 days until sufficiently large colonies were seen (>50 cells per colony). The colonies were stained with 0.5% methylene blue and counted manually using a digital Counter-Pen (Cole-Parmer). Colony formation efficiency following treatment was normalized to the relevant control. To generate the radiation dose–response curves, the data were fitted to the linear quadratic (LQ) model as in equation A:

\[ S(D) = e^{-aD - bD^2} \]  

where \( S(D) \) is the fraction of cells surviving a dose of \( D \) and \( \alpha/\beta \) are inactivation constants. The sensitizer enhancement ratios (SER) for olaparib at 10% were calculated according to equation B:

\[ \text{SER}_{10} = \frac{D_{10(\text{no
drug})}}{D_{10(\text{olaparib})}} \]  

Both parameters, LQ and SER10, were calculated by using GraphPad Prism 5.0 (GraphPad Software).

Western blotting

Calu-6 and A549 cells were seeded at 1 × 106 cells and allowed to attach overnight. Olaparib was added to the cells at a 5 μmol/L concentration 2 hours before exposure to 2 Gy. The lysates were collected immediately and 22 hours after radiation treatment, and equal amounts of protein (10 μg) were resolved in a 10% SDS-PAGE gel and immunoblotted as previously described (31). Membranes were probed for 2 hours at room temperature with mouse antihuman PARP 1:1,000 (BD Pharmingen) and 45 minutes at room temperature with mouse antihuman β-actin 1:80,000 (Sigma).

Immunofluorescence and γ-H2AX signal quantification

Calu-6 cells were seeded at 1 × 106 cells and allowed to attach overnight. Cells were treated with 1 and 5
μmol/L of olaparib 2 hours before exposure to 1, 2, and 4 Gy and returned to standard culture conditions. For detection of γ-H2AX foci, cells were fixed 1 hour and 22 hours after radiation treatment and permeabilized with 1% Triton-X in PBS for 3 minutes at room temperature. Cells were blocked with 8% bovine serum albumin (BSA) for 1 hour at room temperature and incubated with mouse antihuman γ-H2AX 1:500 (Millipore) for 2 hours at 37°C. Cells were then washed and incubated with 1:1,000 Alexa Fluor 488 goat antimouse (Invitrogen) for 1 hour at room temperature in the dark. Coverslips were mounted on a microscope slide with Vectashield mounting medium with 4',6-diamidino-2-phenylindole (DAPI; Vector Labs) and cells visualized in a fluorescence microscope at magnifications of ×40 (Supplementary Fig. S1) and ×100. Images were acquired with a CoolSNAP camera and Metamorph imaging software with a constant exposure of 1,000 ms. Fluorescent signal intensity was analyzed from 100 nuclei per sample from 4 random fields, using MacBiophotonics Image J.

**Calu-6 tumor xenografts**

Animal study protocols were approved by the Institutional Ethics Committee and the Home Office (project license 40/3212) and designed in accordance with the Scientific Procedures Act (1986) and the 2010 guidelines for the welfare and use of animals in cancer research (32). Calu-6 cells in exponential phase were prepared at a concentration of 2 × 10^7 cells/mL in a 1:1 mix of serum-free RPMI 1640 medium and Matrigel (phenol red-free; BD Biosciences). To initiate tumor xenografts, 0.1 mL of cell suspension was implanted intradermally on the back of 10 to 12 weeks old female nu/nu CBA mice. Mice were formed their volumes were measured daily by the main tail artery on the ventral side exposed. The artery weights were monitored daily, and once palpable tumors were formed their volumes were measured daily by the formula: tumor width × length × depth.

**In vivo treatment schedule**

Mice bearing 220 to 250 mm³ tumors were randomized into 4 treatment groups (n = 5): (i) vehicle control (10% DMSO in PBS/10% 2-hydroxy-propyl-β-cyclodextrin daily for 5 days by oral gavage), (ii) olaparib (50 mg/kg daily for 5 days by oral gavage), (iii) 10 Gy fractionated radiotherapy (2 Gy daily for 5 days), (iv) olaparib and 10 Gy (5 × 2 Gy) fractionated radiotherapy (with olaparib given 30 minutes before each daily 2 Gy dose of radiation). Tumor volume measurements were determined daily until they reached 1,000 mm³. The number of days for each individual tumor to quadruple in size from the start of the treatment (relative tumor volume × 4; RTV4) was calculated for the individual tumors in each group.

**Dorsal window chamber model**

Dorsal window chambers (DWC) for dynamic vascular studies using intravital microscopy (IVM) were set up as previously described (33), to assess the effect of radiation and/or olaparib. Treatments were initiated when substantial vascularization was visualized within the tumor (~10 days after implantation of 5 × 10⁶ Calu-6 cells). At the time of imaging, mice carrying window chambers were anaesthetized and the background fluorescence recording read. For vascular perfusion measurements, BSA tagged with an Alexa Fluor 647 (Molecular Probes, Invitrogen) was prepared at 1 mg/mL in sterile saline solution and injected intravenously (0.1 mL/mouse) via the tail vein. When the fluorescence of BSA-Alexa reached a plateau (usually 2–10 minutes after administration), mice were given vehicle or olaparib (50 mg/kg orally). The changes in fluorescence pretreatment and posttreatment with vehicle or olaparib were recorded in real time for a minimum of 80 minutes and analyzed by using a Metamorph Software analysis package. Repeat imaging was carried out on days 1, 3, and 5 of the fractionated drug/radiation treatment protocol, illustrated in Table 1.

**Rat tail artery assay**

Male albino Wistar rats (Harlan UK Ltd.) ages 8 to 12 weeks were euthanized by CO₂ asphyxiation, the tail was removed at the most proximal point and the main tail artery on the ventral side exposed. The artery

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**Table 1.** Schedule of the treatments and window imaging on days 1, 3, and 5 before and after treatment with vehicle and olaparib with or without radiation

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NOTE: In the combination treatments, the mice were irradiated with 2 Gy on day 0, and vehicle and olaparib were given 24 hours later on day 1 at the time of imaging. Once imaging was finished, the mice were given the next fraction (2 Gy) of radiation.

*Real-time IVM imaging.
was constantly covered in oxygenized 37°C Krebs’ solution to prevent dehydration. The wide end of a P2 pipette tip was cut off and the fine end was carved into a fine tip 45° using a scalpel so that it could be used as a cannula for insertion into the artery. The artery was pushed over the cannula until there was an overlap of approximately 5 mm and secured with surgical thread. The artery was cut from the vascular bed to a length of approximately 10 mm. The cannula was then attached to perfusion apparatus, and the artery perfused in a bath of oxygenized (95% O2/5% CO2) Krebs’ solution at 37°C. A total of 3 sections of artery were attached to the perfusion apparatus simultaneously. The arteries were perfused at an initial rate of 0.25 mL/min, which was increased to a maximum of 2 mL/min and allowed to equilibrate for 1 hour. To preconstrict the arteries to approximately 50%, 20 μmol/L phenylephrine (PE; Sigma) in Krebs’ solution was used before the addition of serial dilutions of olaparib or nicotineamide to confirm artery responsiveness. Constriction or dilation of the arterial sections was detected by an increase or decrease in pressure generated by water column displacement, using force transducers connected to a PowerLab/8e software system (ADInstruments Pty Ltd.) and visualized on a computer monitor (Supplementary Fig. S2). Tissue viability and responsiveness were confirmed at the end of each experiment by flushing the artery with Krebs’ solution and reconstituting with 20 μmol/L PE.

**Statistical analysis**

GraphPad Prism 5.0 was used for statistical comparison between 2 groups by a Student’s 2-tailed t test and between more than 2 groups by ANOVA. All data were expressed as mean ± SE.

**Results**

**Inhibition of PARP-1 by olaparib sensitizes NSCLC cell lines to radiation therapy**

Olaparib has previously been shown to enhance the effect of radiation in glioblastoma multiforme cells in vitro (18). Here, we set out to investigate the effects of olaparib on radiation-induced cytotoxicity in 2 NSCLC cell lines (Calu-6 and A549). In Calu-6 cells, we showed that exposure to olaparib for 24 hours at a concentration of 1 μmol/L did not cause significant cytotoxicity, although toxicity was observed at a higher concentration (Fig. 1A). A549 cells were more resistant to both radiation and olaparib. No significant toxicity was observed following 24-hour exposure to 1 or 5 μmol/L olaparib. Toxicity to olaparib was increased when both Calu-6 and A549 cells were exposed continuously to the inhibitor. This is consistent with previous reports, suggesting that PARP inhibition promotes replication-dependent conversion of endogenously arising SSBs into more cytotoxic DSBs (18, 30). Poly(ADP) ribosylation was inhibited by olaparib at the time of irradiation and 22 hours after irradiation in both cell lines (Fig. 1B). Activated PARP is shown as a smear up the gel from 116 kDa due to the ribosylation effect. In the presence of olaparib, no ribosylation occurs and PARP can be visualized as a clean, tight band on the blot. Clonogenic survival curves were also established to examine the effect of PARP-1 inhibition on the response of both Calu-6 and A549 cell lines to radiation treatment (Fig. 1C). In both cell lines, olaparib potentiated the cytotoxicity of radiation treatment where the SER10 at 1 and 5 μmol/L were 1.5 and 1.8 for Calu-6 cell line and 1.3 and 1.6 for A549 cell line.

Many PARP inhibitors have been shown to compromise the repair of DNA SSBs and consequently increase DSBs following treatment of cells with radiation (17, 34). Measurement of γH2AX foci has been adopted as a quick and robust technique to quantify unrepaired DNA DSBs (35). To determine whether olaparib affected DSB repair, Calu-6 cells were fixed at 1 or 22 hours after 0, 1, 2, and 4 Gy irradiation with or without 1 and 5 μmol/L of olaparib and stained for γH2AX foci (Fig. 1D). Fluorescence images were captured and γH2AX foci quantified as illustrated. In the radiation treatment alone and olaparib as a single agent, γH2AX foci levels increased in a dose-dependent manner. In the combination treatments, when γH2AX foci were measured 1 hour after irradiation, the effects on DSBs were simply additive. However, after 22 hours, when the radiation-treated nuclei dropped to nearly background levels, the olaparib-treated nuclei exhibited significantly high levels of γH2AX foci in a dose-dependent manner. Given the number of foci present 24 hours after combination treatment, it is suggested that PARP-1 inhibition by olaparib not only delays the repair of radiation-induced DSBs, but also increases the conversion of endogenous SSBs into DSBs in nonirradiated samples.

**Combination of olaparib with fractionated radiotherapy increases tumor growth delay in Calu-6 tumor xenografts**

We next examined the effect of olaparib alone or in combination with fractionated radiotherapy in a Calu-6 xenograft model. Olaparib alone had no significant effect on the growth of this tumor model compared with vehicle control (P = 0.4). However, when combined with the 5 × 2 Gy fractionated treatment, there was a considerable extension of tumor growth delay compared with radiation alone (Fig. 2A). A significant time increase to RTV4 (Fig. 2B) of approximately 10 days was observed in the combination treatment, compared with 5 fractions of 2 Gy (P = 0.007). A preliminary experiment in an A549 xenograft model was also carried out, and a similar sensitization in the combination treatment was observed (Supplementary Fig. S3A and B). No toxicity, as measured by change in body weight, was observed in the mice treated with olaparib alone or combination treatment.
Figure 1. Effect of olaparib and/or radiation in NSCLC cell lines. A, clonogenic survival of exponential phase Calu-6 and A549 cells exposed to olaparib for 24 hours or continuously for the duration of the assay. B, Western blot analysis of PARP activity in Calu-6 and A549 cells treated with olaparib 2 hours before and 22 hours after radiation. Activated PARP is shown by smearing up the gel from 116 kDa due to the ribosylation effect. In the presence of olaparib, PARP can be visualized as a clean tight band on the blot. C, radiation dose–response curves of Calu-6 and A549 cells treated with vehicle or the indicated concentrations of olaparib 2 hours before and 22 hours after irradiation. The mean surviving fraction ± SE was plotted. D, Calu-6 cells were treated with 1 and 5 μmol/L of olaparib 2 hours before exposure to 1, 2, and 4 Gy. Cells were fixed 1 and 22 hours after irradiation and fluorescence intensity of the γH2AX signal determined. Mean values of 100 nuclei ± SE are presented, and all data were derived from at least 3 independent experiments. *; P < 0.01.
Olaparib increases vascular perfusion in Calu-6 tumors established in a DWC model

New generation PARP inhibitors have been reported to enhance the effects of chemotherapeutics and radiation, not only through DNA repair but also by affecting tumor vasculature; via a mechanism that supports the notion of increased tumor oxygenation and improved drug delivery (19, 21). In these studies, changes in vascular perfusion were measured only after a single dose of PARP inhibitor. To help interpret the radiosensitizing effects of olaparib using a clinically relevant fractionation schedule, we measured vascular perfusion in Calu-6 tumors implanted into DWCs at various times during fractionated treatment. Details of the treatment schedule are given in Table 1: imaging of tumors was carried out on days 1, 3, and 5, before and after treatment with vehicle or olaparib, both with or without radiation. Figure 3A shows the typical fluorescence intensity profiles when mice are given the BSA-Alexa followed by treatment with vehicle or olaparib. Administration of the BSA-Alexa results in a rapid increase in fluorescence intensity which reaches a plateau and is maintained for 75 minutes postinjection. Administration of olaparib as a single agent (top) or in combination with radiation (bottom) results in an increase in fluorescence intensity in the Calu-6 tumors. A second plateau is reached following drug treatment and this change in fluorescence can be interpreted as an increase in tumor vessel perfusion. In the examples given in Fig. 3A, this equates to a 1.3- and 1.4-fold change in vessel perfusion in the nonirradiated and irradiated tumors, respectively. Figure 3B shows the olaparib-induced enhancement of tumor perfusion following 1×, 3×, or 5× doses of drug with or without radiation treatment. The effect of olaparib was significantly more dramatic on day 1, particularly in the combination treatment with radiation (P = 0.01) and was maintained during the fractionated schedule. Figure 3C shows the distribution of BSA-Alexa on tumor vessels in all 4 treatment groups during a real-time imaging period of 60 minutes. Interestingly, in the combination treatment it is possible to see some of the existing tumor vessels opening after olaparib administration (Fig. 3C, white arrows). Previous findings have shown that a single dose of nicotinamide and the PARP-1 inhibitors AG14361 and AG014699 also have the ability to increase tumor vessel perfusion in SW620 and HT29 tumors. In this study, we have shown that in Calu-6 tumors, a single dose of olaparib allows the tumor vessels to be more perfused just before radiation treatment, and this is maintained during the treatment period (5 days). In Supplementary Fig. S4A and B, we show that we obtained identical effects on tumor perfusion when nicotinamide is given to mice harboring Calu-6 tumors, although the dose of nicotinamide used to achieve the effect is 20× higher than that of olaparib. This suggests that the underlying mechanism by which these 2 agents are acting may be similar, allowing the tumor to be more oxygenated before each radiation fraction.

Olaparib causes relaxation of preconstricted rat tail artery

Previous reports have also shown nicotinamide to reduce spontaneous rhythmic artery contractions in an ex vivo rat tail artery assay (36–38). As olaparib had a marked effect in the tumor vessel perfusion of the Calu-6 xenograft, the effect of this drug in preconstricted rat tail arteries was examined. Following artery precontraction with PE, olaparib or nicotinamide was administered with PE, and the effect was recorded for 30 minutes (Fig. 4A). Both olaparib and nicotinamide dilated PE preconstricted rat tail artery ex vivo in a dose-dependent manner (Fig. 4B). However, olaparib was approximately 30-fold more potent at inducing this effect, as a 50% relaxant activity (EC50) was achieved.
with 5 mmol/L nicotinamide whereas with olaparib it was achieved at 150 µmol/L olaparib.

Discussion

Radiation therapy is used widely in the treatment of cancer and is curative in a number of settings. However, there may still be opportunities to augment the effectiveness of radiotherapy by overcoming resistance mechanisms such as tumor hypoxia or repair of damaged DNA. Here, we show that the PARP inhibitor olaparib sensitizes NSCLC to radiation therapy by compromising the repair of DNA. In addition, olaparib treatment increases tumor vascular perfusion, which may also be beneficial to drug delivery and tumor oxygenation.

PARP inhibitors, such as olaparib, have been found to have monotherapy activity against tumor cells harboring BRCA1 or BRCA2 mutations, through a synthetic lethality interaction (25, 39, 40). Cancer cells with a compromised homologous recombination (HR) pathway, such as in BRCA deficiency, become highly dependent upon PARP activity for maintenance of genomic integrity and survival (39, 41). There are currently 8 different PARP inhibitors undergoing clinical trials (14) and while the activity of these agents is being explored in tumors with HR deficiency, their potential to enhance other therapies such as radiotherapy,
irrespective of tumor HR status, remains to be explored in detail.

Although 4 PARP inhibitors (Fig. 5; AG14361, ABT-888, GPI-15427, and E7016) have been reported to enhance the response to radiation in various tumor models (17, 19, 20, 34, 42), olaparib has only been shown to potentiate the radiation response in glioblastoma cells in vitro and in cells deficient in HR or nonhomologous end joining (18, 30). Here, we provide the first report showing that olaparib increases the radiosensitivity of NSCLC cells both in vitro and in vivo.

Calu-6 cells treated with olaparib alone for 24 hours showed a reduction in PARP activity as measured by Western blot analysis. After treatment with olaparib, a slight increase in γH2AX foci was observed in a dose-dependent manner indicating that un repaired SSBs from internal agents such as reactive oxygen species or products of lipid peroxidation were converted into DSBs at the time of replication. However, the effect of PARP inhibition was more profound when cells were exposed to olaparib for longer. It was observed that at 22 hours there was an increase in DSBs, and in the combination

![Figure 4. Olaparib caused relaxation in the ex vivo PE preconstricted rat tail artery. A, representative histogram of the dilatory effect of 500 μmol/L olaparib in 10 μmol/L PE preconstricted rat tail artery. B, dose–response of olaparib and nicotinamide in a PE preconstricted rat tail artery. Each assay was run with 3 arteries, and the experiment was carried out in triplicate.](image)

![Figure 5. Structures of olaparib, AG14361, ABT-888, and GPI-15427, a related compound of E7016.](image)
treatment there was still a considerable number of residual DNA DSBs compared with radiation alone. These observations are consistent with previous studies where inhibition of PARP activity is thought to delay endogenously arising SSBs and radiation-induced SSBs, by generating collapsed replication forks which can be repaired by HR in HR-proficient cells (18, 30).

In the in vivo experiments, oral administration of olaparib alone, daily for 5 days, did not cause significant delay in tumor growth. However, when combined with radiation, there was a significant enhancement of tumor response. Interestingly, the tumor growth delay observed in the combination treatment was quite similar to the growth delay observed in a fractionated (10 × 2 Gy) treatment, previously published by our group using the same xenograft model (43). Thus, by combining olaparib with fractionated radiation, we were able to reduce the total dose of radiation by nearly half to cause the same effect as a fractionated dose of 20 Gy.

Recently, at least 2 new generation PARP inhibitors (AG014699 and AG14361) have been reported to have vasoactive properties, and AG14361 has been shown to enhance the response to radiation (19, 21). Nicotinamide, which can increase tumor oxygenation, has also undergone clinical evaluation (44). It is structurally related to the 3-substituted benzamides (first generation PARP inhibitors) and has been extensively studied for its ability to sensitize tumor cells to radiation therapy in vivo. The mechanism of action of nicotinamide is to prevent intermittent vascular shutdown in tumors, and the new generation PARP inhibitors, including olaparib, are all structurally related to nicotinamide (Fig. 5; refs. 23, 45, 46). Therefore, to determine whether olaparib has similar vasoactive properties to nicotinamide, we have used a well-established technique to look at Calu-6 tumor vessel perfusion in real-time by IVM in a DWC. The effects on tumor vasculature were confirmed not only by monitoring the distribution of BSA-Alexa, as an indicator of permeability/perfusion, but also by looking at the vasoconstriction/vasorelaxant effect, using an ex vivo rat tail artery assay. Although tumor vessels have fewer smooth muscle cells and are less sensitive to PE vasoconstrictive effects, nicotinamide has been previously reported to be as equally effective in modifying blood flow in tumors and their supplying arteries (38). Therefore, a major finding of this work is that olaparib is a more potent vasorelaxant than nicotinamide, and its effects are maintained during treatment with drug alone and when drug and radiation are combined in a fractionated treatment schedule.

In conclusion, although olaparib has so far shown potency in HR-deficient (BRCA-deficient) tumors, this PARP inhibitor will also sensitize tumor cells to DNA-damaging agents such as radiation. Here, we show that olaparib enhances the effect of radiotherapy both in vitro and in vivo in an NSCLC model that is HR proficient. This evidence suggests that olaparib should be considered as a promising drug candidate for combination with radiotherapy for the treatment of NSCLC.

Disclosure of Potential Conflicts of Interest

S.R. Wedge and M.J. O’Connor are full-time employees and stockholders of AstraZeneca plc. I.J. Stratford received a commercial research grant from AstraZeneca.

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