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The Suppression of DNA Repair Induced by PARP-1 Inhibitors Rucaparib and Olaparib in Combination with the Radiopharmaceutical ^{131}I-MIBG in Noradrenaline Transporter-Expressing Xenograft Tumors

Donna L Nile[1*], Colin Rae[1], Colin Nixon[2], Mark N Gaze[3] and Robert J Mairs[1]

[1]*Radiation Oncology, Institute of Cancer Sciences, University of Glasgow, United Kingdom*

[2]*Beatson Institute for Cancer Research, University of Glasgow, United Kingdom*

[3]*University College London Hospitals, United Kingdom*

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[*]Correspondence Address: Donna Nile, Radiation Oncology, Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom, Tel: 0141 330 4129; Email: Donna.Nile@glasgow.ac.uk

Abstract

Radioiodinated meta-iodobenzylguanidine (^{131}I-MIBG) is an effective treatment for tumors that express the noradrenaline transporter (NAT); including neuroblastoma, phaeochromocytoma, and gut neuroendocrine tumors. We have previously shown *in vitro* that the efficacy of ^{131}I-MIBG was enhanced following its combination with PARP-1 inhibitors, rucaparib or olaparib. In order to assess *in vivo* the therapeutic benefit of combined radiotherapy and PARP-1 inhibition, we have first established a reliable protocol for the administration of olaparib and rucaparib, in NAT-expressing tumour xenografts. PARP-1 inhibition in combination with ^{131}I-MIBG therapy was well tolerated with limited toxicity. Furthermore, administration of both PARP-1 inhibitors as single agents inhibited DNA repair in a time-dependent manner. These preliminary results highlight the therapeutic potential of PARP-1 inhibitors *in vivo*, and indicate the feasibility of combining rucaparib or olaparib with ^{131}I-MIBG for the treatment of neuroendocrine tumors in preclinical models.

Keywords: Neuroblastoma; ^{131}I-MIBG; PARP-1

Abbreviations: H&E: Haematoxylin and Eosin; HPBCD: 2-hydroxylpropyl-β-cyclodextrin; ^{131}I-MIBG: Iodine-131 Metaiodobenzylguanidine; MBq: Megabecquerel; NAT: Noradrenaline Transporter; PARP-1: Poly(ADP-ribose) Polymerase-1; PBS: Phosphate Buffered Saline

Introduction

Radioiodinated meta-iodobenzylguanidine (^{131}I-MIBG) is an effective treatment for the primordial neural crest-derived paediatric tumor, neuroblastoma [1-3]. A structural analogue of the catecholamine neurotransmitter, noradrenaline, ^{131}I-MIBG is actively accumulated by tumors expressing the noradrenaline transporter (NAT) including neuroblastoma, the adrenal tumors phaeochromocytoma and paraganglioma [4], neuroendocrine tumors of the gut [5] and medullary thyroid carcinoma [6]. Whilst effective when administered as single agent therapy [2-4], maximal therapeutic benefit from ^{131}I-MIBG will be obtained by its combination with chemotherapy [7]. Poly (ADP-ribose) polymerases (PARPs) mediate the post-translational modification of target proteins, thus signalling the site of DNA damage and initiating DNA repair. Indeed, PARP-1 inhibition was shown to exhibit synthetic lethality in BRCA-deficient cells [8,9], and has since proven an effective treatment in cancers that are deficient in BRCA-mediated DNA repair [10,11]. olaparib and rucaparib are FDA-approved PARP-1 inhibitors undergoing Phase II/III clinical evaluation [10-12].

We recently demonstrated that treatment with rucaparib or olaparib significantly reduced the IC_{50} dose of X-radiation or ^{131}I-MIBG therapy required to kill neuroblastoma or NAT-expressing glioma cells [13]. We hypothesized that the PARP-1 inhibitors effectively lowered the threshold for radiation-induced cell death, by significantly delaying repair of radiation-induced DNA damage, and promoting cell cycle arrest. Our purpose is now to assess [1] whether PARP-1 inhibition aggravates ^{131}I-MIBG toxicity [2] whether enhancement of the prolongation of DNA damage by PARP-1 inhibition also obtained *in vivo*.

Methods

Establishment and treatment of xenografts

In vivo experiments were performed in accordance with the Animals (Scientific Procedures) Act 1986. This research was reviewed and approved by the University of Glasgow's Ethical Review Board. Tumors derived from UVW glioma cells expressing NAT [14] were produced by subcutaneous injection of $3x10^6$ cells into 6 week old female, athymic CD1 nude (*nu/nu*) mice (Charles Rivers plc, Kent, UK) as described previously [15]. Experimental therapy was initiated 3-5 weeks after injection, once the tumor volume had reached $60mm^3$. rucaparib (1 or 10mg/kg), olaparib (30mg/kg), or vehicle (10% v/v DMSO, 10% v/v HPBCD, 2-hydroxylpropyl-β-cyclodextrin, in PBS) were intraperitoneally injected. Tumors were excised 2, 6, or 24h after injection.

Assessment of Treatment Toxicity

^{131}I-MIBG (10 MBq) was administered by intraperitoneal injection to 9 week old female, athymic CD1 nude (*nu/nu*) mice. For combination therapy, mice were also intraperitoneally injected with rucaparib (10mg/kg), olaparib (30mg/kg), or vehicle (10% v/v DMSO, 10% v/v HPBCD in PBS) at the same time as ^{131}I-MIBG administration, and for the next two consecutive days. Mice were weighed 3 times weekly and monitored daily to assess treatment toxicity.

Immunohistochemistry

Immunohistochemical analysis was performed on formalin-fixed paraffin-embedded 4µm sections of tumors according to standard protocols. Primary antibodies used were anti-γH2AX (1:50; Cell Signalling Technology, The Netherlands; 9718) and anti-Ki67 (1:100; Thermo Scientific, UK; RM-9106) after citrate buffer and antigen retrieval (25min, 98 °C) on a Pre-Treatment (PT) Module (Thermo Scientific, UK). All staining was performed on a Dako Autostainer Link 48 platform (Dako, UK). Slides were digitized using the SCN400F scanner (Leica Biosystems, UK) at x20 magnification and visualized using the Slide Path Digital Image Hub, version 4.0.1 (Leica Biosystems, UK).

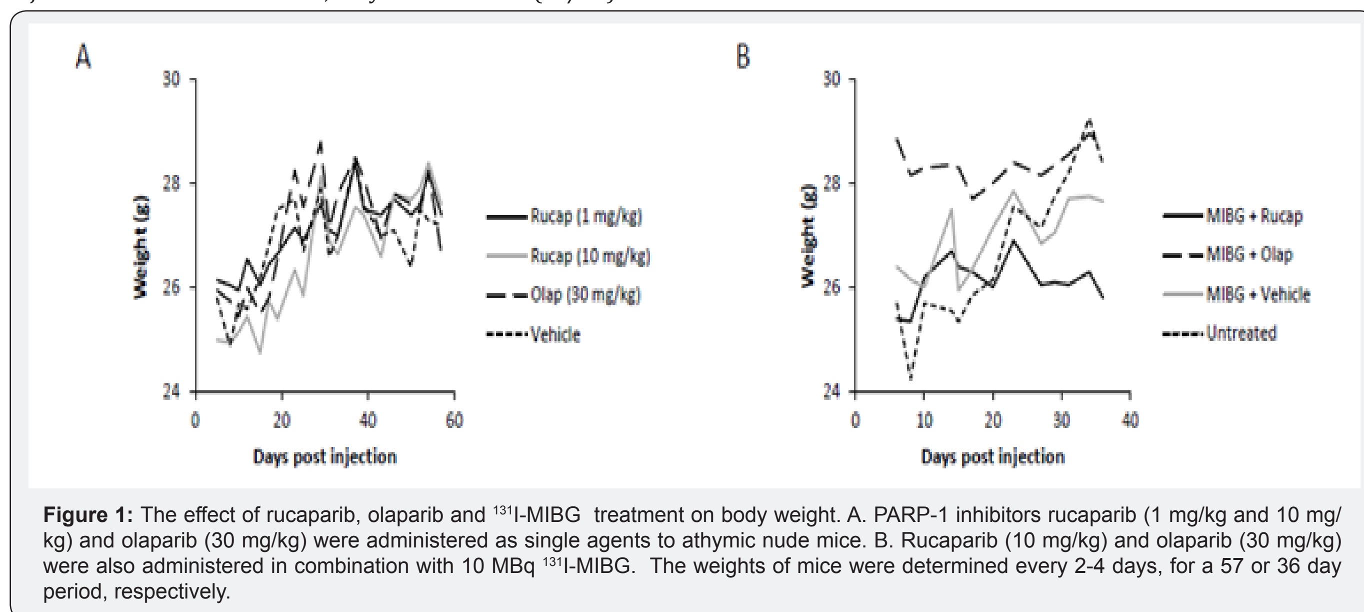

Figure 1: The effect of rucaparib, olaparib and ^{131}I-MIBG treatment on body weight. A. PARP-1 inhibitors rucaparib (1 mg/kg and 10 mg/kg) and olaparib (30 mg/kg) were administered as single agents to athymic nude mice. B. Rucaparib (10 mg/kg) and olaparib (30 mg/kg) were also administered in combination with 10 MBq ^{131}I-MIBG. The weights of mice were determined every 2-4 days, for a 57 or 36 day period, respectively.

Results

No adverse toxic effect was observed following the administration of 1mg/kg or 10mg/kg rucaparib. Mice continued to gain weight for up to 57 days post injection, at a rate comparable to the tumors in the vehicle control group of mice. Negligible toxicity was also observed after treatment with 30mg/kg olaparib (Figure 1A). Similarly, mice maintained weight for up to 36 days after treatment with 10mg/kg rucaparib or 30mg/kg olaparib in combination with 10 MBq ^{131}I-MIBG (Figure 1B). Therefore it was concluded that the administration of PARP-1 inhibitors did not aggravate ^{131}I-MIBG toxicity.

We previously demonstrated that PARP-1 inhibitors, administered in combination with external beam radiation, promoted substantial DNA damage 2h after administration *in vitro* [13]. Moreover, this radiation-induced DNA damage persisted for 24h following treatment with PARP-1 inhibitors. We therefore resolved to determine the significance of delayed restitution of DNA *in vivo*. Tumor xenografts were excised 2, 6 and 24h after PARP-I inhibitor injection. Fixed, paraffin-embedded tumor sections were then stained with haematoxylin and eosin (H&E), anti-Ki67 or anti-γH2AX antibodies to observe gross tumor histology, cell proliferation and DNA damage, respectively. Exposure to rucaparib failed to affect the gross xenograft tumor histology, however, decreased Ki67-positive staining observed at 2h and 6h after treatment indicates decreased cell proliferation (Figure 2). Negligible DNA damage was apparent 2h and 6h after treatment, manifest by the predominance of counter staining. However, substantial DNA damage was observed 24h after treatment, according to γH2AX-positivity. Similarly, following exposure to olaparib, there was no change in tumor histology, whilst decreased cellular proliferation was observed 6h after

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treatment (Figure 3). Substantial DNA damage was also observed 24h following exposure to olaparib.

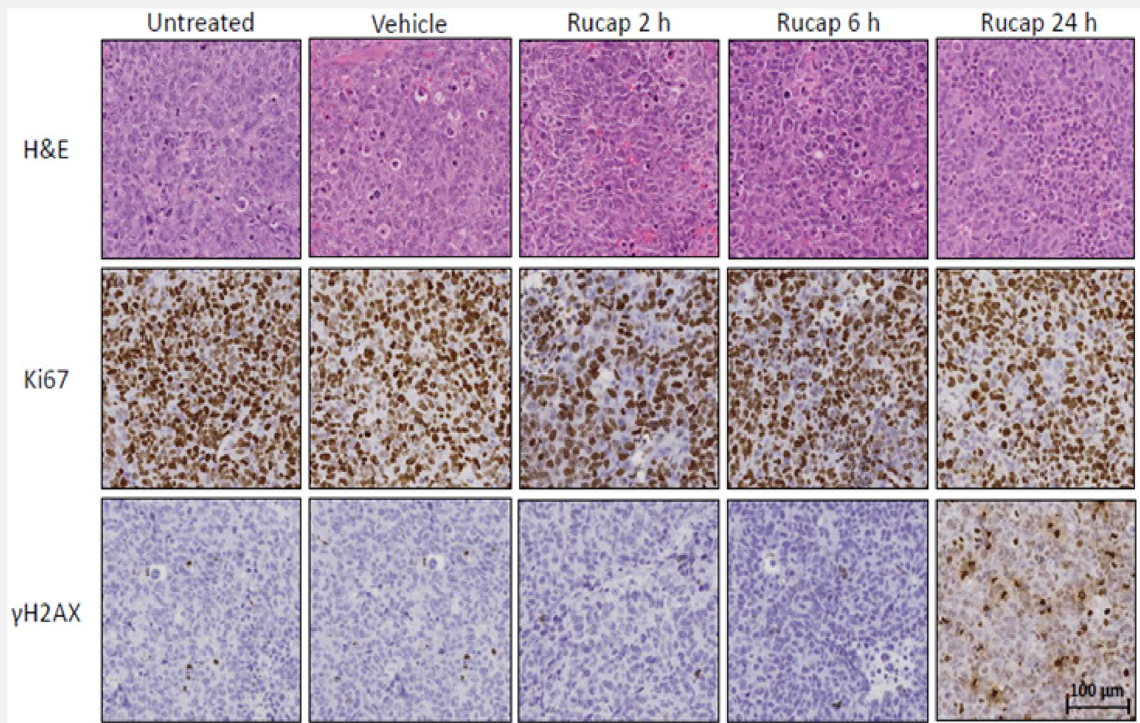

Figure 2: The effect of rucaparib on cell proliferation and DNA damage. Nude mice harbouring UVW/NAT-derived xenografts were intraperitoneally injected with rucaparib (10mg/kg), vehicle control (10% DMSO, 10% HPBCD), or nothing (untreated). Tumors were excised 2-24h after injection. Ki67 and γH2AX staining revealed the extent of cell proliferation and DNA damage, respectively (x20 magnification).

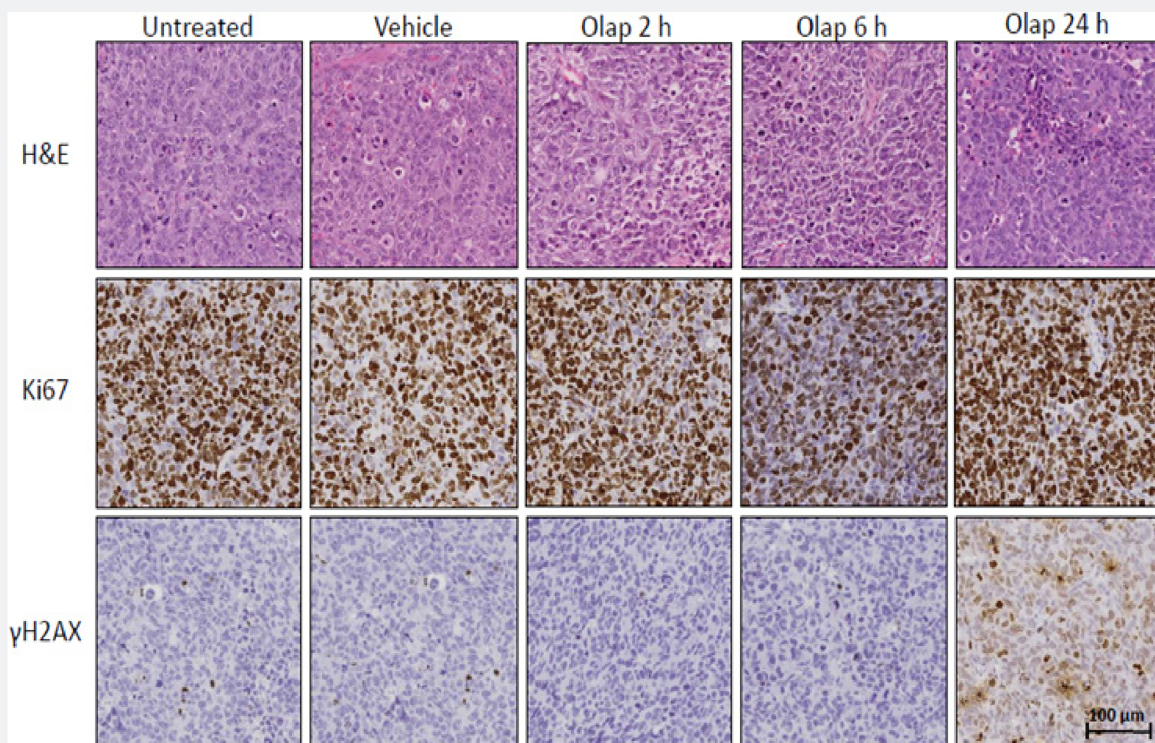

Figure 3: The effect of olaparib on cell proliferation and DNA damage. Nude mice harbouring UVW/NAT-derived xenografts were intraperitoneally injected with olaparib (30mg/kg), vehicle control (10% DMSO, 10% HPBCD), or nothing (untreated). Tumors were excised 2-24h after injection. Ki67 and γH2AX staining revealed the extent of cell proliferation and DNA damage, respectively (x20 magnification).

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Conclusion

The noradrenaline analogue ^{131}I-MIBG selectively accumulates within cells that express the noradrenaline transporter, NAT. This can be exploited therapeutically for the treatment of NAT-expressing tumors such as neuroblastoma, phaeochromocytoma, and gut neuroendocrine tumors. Whilst effective when administered as single agent therapy, maximal therapeutic benefit from ^{131}I-MIBG is expected to be obtained by its combination with chemotherapy.

Activation of the PARP-1 DNA repair pathway follows genotoxic insult. We have previously shown that PARP-1 inhibition, following treatment with PJ34 [16] or rucaparib or olaparib [13], enhanced the antitumor efficacy of ^{131}I-MIBG in NAT-expressing neuroblastoma and glioma cells. We hypothesized that PARP-1 inhibition effectively lowered the threshold for radiation-induced cell death, following the delayed repair of radiation-induced DNA damage, which prompted G2/M cell cycle arrest. Our current aim was to determine whether prolonged DNA damage by inhibition of PARP-1 also obtained *in vivo*. We observed that PARP-1 inhibitor single agent treatment prevented DNA repair in tumor xenografts in a time-dependent manner. γH2AX staining, which is indicative of DNA double strand breaks, was observed at 24h after administration of rucaparib or olaparib, but not at earlier time points. Jiang *et al*. [17] also demonstrated that olaparib single agent treatment significantly increased the number of γH2AX foci 24h after treatment in non-small cell lung cancer xenografts. Interestingly, the DNA damage sustained was significantly enhanced following combination of 50mg/kg olaparib with 10Gy irradiation, particularly under hypoxic conditions [17].

The measurement of PARP-1 enzymatic activity in peripheral blood leukocytes has been used in clinical trials of rucaparib [12,18] and olaparib [19,20] as a pharmacodynamic biomarker. However, recent demonstration of poor correlation between leukocyte PARP-1 activity and the anti-tumor efficacy [18,19] suggest that this assay may be suboptimal. Therefore, alternative biomarkers, such as the γH2AX or RAD51 foci, may be more reliable indices of effectiveness [21].

Using a murine preclinical model [15,16,22], we have established an effective treatment regimen for the administration of olaparib or rucaparib in combination with ^{131}I-MIBG. The treatment procedure, which mimics therapy schedules used clinically, did not aggravate the toxicity of either agent administered alone. This was manifest by the maintenance of murine body weight up to 57 or 36 days following administration of PARP-1 inhibitors as single agents or in combination with ^{131}I-MIBG, respectively. Similarly, Min *et al.* [23] demonstrated no significant reduction in mouse weight compared to vehicle control mice, following administration of 50mg/kg olaparib. The drug concentrations used in this study are comparable to those used previously in experimental therapy of mice bearing xenografts derived from neuroblastoma, medulloblastoma and gastric cancer [23-25]. Furthermore, the administered doses are below the 400mg twice daily maximum tolerated dose of olaparib [10] and the 600mg twice daily recommended dose of rucaparib [18].

The results of this *in vivo* study have demonstrated that administration of PARP-1 inhibitors, rucaparib and olaparib, as single agents, inhibited DNA repair in mouse tumor xenografts in a time-dependent manner. This finding is in support of our published *in vitro* data [13]. We also established a reliable protocol for the administration of olaparib and rucaparib in NAT-expressing tumor xenografts established in athymic mice. The doses administered were similar to those used clinically, and were well tolerated. These preliminary results indicate the feasibility of combining rucaparib or olaparib with ^{131}I-MIBG for the treatment of neuroendocrine tumors. It is expected that the combination of PARP-1 inhibition with ^{131}I-MIBG will engender more effective therapy of NAT-expressing tumors.

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