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

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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-hydroxypropyl-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-hydroxypropyl-β-cyclodextrin, in PBS) were intraperitoneally injected. Tumors were excised 2, 6, or 24h after injection.

Assessment of Treatment Toxicity

131I-MIBG (10 MBq) was administered by intraperitoneal injection to 9 week old female, athymic CD1 nude (nu/nu) mice.

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 131I-MIBG (Figure 1B). Therefore it was concluded that the administration of PARP-1 inhibitors did not aggravate 131I-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...
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).
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, pheochromocytoma, 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 published 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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