Therapeutic Efficacy of a Bivalent Inhibitor of Prostate-Specific Membrane Antigen Labeled with Copper-67

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Disclosure

E. M. van Dam and M. J. Harris are employed by Clarity Pharmaceuticals, the licensee of relevant intellectual property. P. S. Donnelly and N. A. Zia are inventors of intellectual property, licensed from the University of Melbourne to Clarity. P. S. Donnelly serves on the Scientific Advisory Board of Clarity and has a financial interest. Unrelated to this project, Prof. Hicks has shares in Telix Radiopharmaceuticals with proceeds donated to his institution. No other potential conflict of interest relevant to this article was reported.

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Running title: Radionuclide Therapy with ⁶⁷CuSarbisPSMA

Abstract

Radionuclide therapy targeting prostate-specific membrane antigen (PSMA) is a promising treatment for prostate cancer. We reported a ligand featuring two lysine-ureido-glutamate groups, ^{64}Cu -CuSarbPSMA previously. Here, we report the therapeutic potential of ^{67}Cu -CuSarbPSMA. **Methods:** Growth of PSMA-positive xenografts was evaluated following treatment with ^{67}Cu -CuSarbPSMA or ^{177}Lu -LuPSMA I&T. **Results:** At 13 days post-injection, tumor growth was similarly inhibited by the two tracers in a dose-dependent manner. Survival was comparable after single (30 MBq) or fractionated administrations (2 x 15MBq, two weeks apart). **Conclusion:** ^{67}Cu -CuSarbPSMA is efficacious in a PSMA-expressing model of prostate cancer.

Keywords: copper-64; copper-67; theranostics; prostate cancer; prostate specific membrane antigen.

Introduction

Prostate-specific membrane antigen (PSMA) is a membrane-bound enzyme which can act as a glutamate carboxypeptidase or folate hydrolase. In prostate cancer cells, it becomes membrane-bound and overexpressed with androgen independence and metastasis (1), making it a promising target for both imaging and therapy (2). Radiolabeled peptidomimetic inhibitors of PSMA containing a lysine-ureido-glutamate functional group are effective tracers for imaging prostate cancer using positron emission tomography (PET) (2-4). A theranostic paradigm involves PET with Glu-NH-CO-NH-Lys-(Ahx)-(HBED-CC) labelled with gallium-68 ($t_{1/2} = 68$ min, $E_{\beta^+}(\text{mean}) = 0.89$ MeV) (^{68}Ga -GaPSMA-11) to guide therapy with ^{177}Lu -LuPSMA-617 (^{177}Lu , $t_{1/2} = 6.65$ d, $\beta^- = 100\%$, $E_{\beta^-}(\text{mean}) = 134$ keV) (5-7). This approach has allowed personalized treatment of advanced prostate cancer but the short half-life of gallium-68 limits the ability to do prospective dosimetry (8) as does the use of differing ligands for diagnosis and therapy. The DOTAGA-containing urea-based PSMA inhibitor called PSMA I&T, which can be labelled with either gallium-68 or lutetium-177 or the use of ^{68}Ga -GaPSMA-617 in place of ^{68}Ga -GaPSMA-11(5,9,10), can overcome the latter limitation but both approaches remain constrained for prospective dosimetry. Both limitations could be potentially addressed by using the positron-emitting (β^+) copper-64 ($t_{1/2} = 12.7$ h, $\beta^+ = 17.4\%$, $E_{\beta^+}(\text{mean}) = 278$ keV) for diagnosis and β^- -emitting (β^-) copper-67 ($t_{1/2} = 61.9$ h, $\beta^- = 100\%$, $E_{\beta^-}(\text{mean}) = 141$ keV) for therapy. The β^- -emissions of copper-67 have a mean range of 0.2 mm and are appropriate for the treatment of small tumors down to 5 mm in diameter (11). The γ -emission of copper-67 (copper-67: 185 keV 49% and 93 keV 16%) may be beneficial for quantitative single-photon emission computed tomography (SPECT) to verify radiation dose to tumor

and critical organs (12). The efficacy of targeted therapy with copper-67 has been demonstrated previously in non-Hodgkin's lymphoma and neuroendocrine tumors (NET) (11,13,14).

The potential advantages of copper radiopharmaceuticals are dependent on high retention in tumors and clearance from normal tissues. The use of chelators that form copper complexes susceptible to release of copper *in vivo* can lead to high liver uptake at late time-points (15). Importantly, copper(II) complexes of sarcophagine (Sar = 3,6,10,13,16,19-hexa-azabicyclo[6.6.6]icosane) based ligands are stable *in vivo* (16). A Sar derivative conjugated to a somatostatin receptor-targeting peptide, ^{64}Cu -CuSarTATE, allows acquisition of high-quality images at 24 hours post-injection in patients with NET (16), and its therapeutic pair, ^{67}Cu -CuSarTATE, is highly efficacious in a NET model (14). We recently reported high tumor uptake and retention of a copper-64-labeled sarcophagine ligand tethered to two lysine-ureido-glutamate functional groups in a PSMA-positive model (17). Here, we evaluate the therapeutic efficacy of its copper-67 labelled pair, ^{67}Cu -CuSarbisPSMA (Figure 1), in the same PSMA-positive tumor model.

Material and methods

Radiochemistry

Synthesis of ^{67}Cu -SarbisPSMA: ^{67}Cu - CuCl_2 (756 MBq, 70 μL , 0.05 M HCl, ISU, USA) was added to a mixture of SarbisPSMA (AusPep, Australia)(17) (20 μg , 10 nmol, in 10 μL of 50:50 ethanol:water) and sodium phosphate buffer (0.1 M, pH 6.2, 350 μL). After 10 min at room temperature analysis by HPLC indicated $\geq 95\%$ radiochemical purity, (72 GBq/ μmol , $R_t = 10.9$ min, precursor $R_t = 11.0$ min). HPLC conditions: Shimadzu SPD-10ATvP HPLC, Phenomenex Luna C18 100 Å column (4.6 $\times$ 150 mm, 5 μm), 1 mL/min, 5- 100% acetonitrile (0.5% TFA) over 15 min.

^{177}Lu -LuPSMA I&T was prepared according to published procedures in $\geq 95\%$ radiochemical yield: PSMA I&T (200 μg , 0.13 μmol) (ABX, Germany) and $^{177}\text{LuCl}_3$ (8 GBq) (ANSTO, Australia) (58 GBq/ μmol) (5).

In vivo comparative experiment

All animal experiments were performed with the approval of the institutional animal ethics committee. Eight-week old male NSG mice (Australian BioResources, New South Wales) were implanted with LNCaP (human prostate adenocarcinoma) cells as described previously (17). Mice (n=5)

bearing subcutaneous LNCaP xenografts (mean tumor volume $\sim 90 \text{ mm}^3$) were randomized into five groups and injected intravenously with either vehicle (saline), ^{67}Cu -CuSarbisPSMA (5 MBq or 30 MBq) or ^{177}Lu -LuPSMA I&T (5 MBq or 30 MBq) on day 1 of the experiment. Twice weekly monitoring of tumor size and health was performed with mice euthanised when tumor volume (calculated as length $\times$ width $\times$ height (mm) $\times \pi/6$) exceeded 1200 mm^3 .

***In vivo* dose-dependency experiment**

Male NSG mice (n= 8 per group) with subcutaneous LNCaP xenografts (mean tumor volume = $\sim 240 \text{ mm}^3$) were injected with either saline or ^{67}Cu -CuSarbisPSMA (7.5 MBq, 15 MBq or 30 MBq) on day 1 of the experiment. An additional cohort was injected with ^{67}Cu -CuSarbisPSMA (15 MBq) on day 1 and day 15 of the experiment (n = 8) and monitored as above.

Data Analysis

Percentage tumor growth inhibition (TGI) was calculated as $100 \times (1 - \Delta T/\Delta C)$ where ΔC and ΔT were determined by subtracting the mean tumor volume (in the vehicle control and treated groups respectively) on day 1 of treatment, from the mean tumor volume on either day 17 in the comparative experiment or day 13 for the dose-dependency experiment. Statistical analysis was performed using Graph Pad Prism 8.0 (Graph Pad, CA). Statistical comparisons between the vehicle control and treated cohorts were done by a one-way ANOVA followed by a Dunnett's post hoc test. Toxicity was assessed by body-weight loss and physical/behavioural observation. The experiment was ended on day 82 or 85 with the remaining mice censored for survival. Survival (tumor volume $\geq 1200 \text{ mm}^3$) curves were analysed using the Mantel Cox log rank test.

Results

^{67}Cu -CuSarbisPSMA and ^{177}Lu -LuPSMA I&T are efficacious against LNCaP tumor xenograft model

^{67}Cu -CuSarbisPSMA was prepared in high radiochemical purity ($>95\%$) in sodium phosphate buffer without the need for further purification before injection. Mice bearing subcutaneous LNCaP tumors, were randomized into groups of 5 animals (mean tumor volume = 90 mm^3) then injected with either saline, ^{67}Cu -CuSarbisPSMA (5 MBq, 0.06 nmol or 30 MBq, 0.36 nmol) or ^{177}Lu PSMA I&T (5 MBq, 0.08 nmol or 30 MBq, 0.48 nmol). The inhibition of tumor growth was similar for both agents following administration of both 5 MBq and 30 MBq activity levels but demonstrated dose dependency

(Table 1 and Figure 2). Survival was increased significantly in the 30 MBq dose cohorts when compared to the cohorts treated with 5 MBq (5 MBq vs 30 MBq, $P = 0.002$ for both agents) (Figure 2B).

Inhibition of LNCaP tumor growth is dependent on the activity of ^{67}Cu -CuSarbPSMA administered

Mice were inoculated with LNCaP cells and once tumors were established were randomized into 5 groups (mean tumor volume = 240 mm^3) and injected with either saline or increasing doses of ^{67}Cu -CuSarbPSMA (7.5 MBq, $0.21 \mu\text{g}$, 0.1 nmol ; 15 MBq, $0.45 \mu\text{g}$, 0.22 nmol ; or 30 MBq, $0.89 \mu\text{g}$, 0.44 nmol) on day 1 of the experiment *via* intravenous injection. An additional group was injected with ^{67}Cu -CuSarbPSMA (15 MBq, $0.45 \mu\text{g}$, 0.22 nmol) on day 1 and 15 to investigate the potential of fractionated dose protocols. On day 13, tumor growth in each treatment group was suppressed versus the control group (Table 2). All treatments increased survival significantly compared with the vehicle control (7.5 MBq, <0.001 ; 15 MBq, <0.001 ; 30 MBq, <0.001). There was a trend for prolonged tumor growth inhibition and survival in the fractionated dose group ($2 \times 15 \text{ MBq}$) compared to the single dose (30 MBq), although this was not statistically significant (Figure 3).

Discussion

^{64}Cu -CuSarbPSMA has excellent uptake in LNCaP tumors in male NSG mice and importantly showed excellent retention in the tumor up to 24 h post injection (17), suggesting that the copper-67 variant may be suited to PSMA-targeted radiotherapy. In this work we demonstrate that ^{67}Cu -CuSarbPSMA and ^{177}Lu -LuPSMA I&T provide similar tumor inhibition and increases in survival at equivalent administered activities. This is not surprising as the energy from the β^- emissions from copper-67 and lutetium-177 are similar. The shorter half-life of copper-67 compared to lutetium-177 (61.9 h vs 6.7 d) means that repeated dosing might be feasible over a shorter timeframe, potentially providing better control of rapidly repopulating tumors. Administration of two cycles of 15 MBq of activity resulted in similar tumor growth inhibition to a single 30 MBq administration although there was a trend for prolonged tumor growth inhibition in the fractionated dose group, but the difference was not statistically significant. Further studies could investigate the efficacy of administering four cycles of 7.5 MBq of activity. It is pertinent to note that the half-life of copper-67 is similar to that of ^{90}Y (64.6 h) but with a particulate energy similar to that of lutetium-177. How these physical characteristics might influence therapeutic efficacy in lesions of differing size and biology remains to be determined. Future work will include comparative biodistribution studies to quantify tumor uptake and retention to allow estimates of dose to the tumor and normal tissue.

Conclusion

^{67}Cu -CuSarbPSMA is efficacious in the PSMA expressing LNCaP model of prostate cancer and further evaluation of the combination of $^{64/67}\text{Cu}$ -CuSarbPSMA as a theranostic approach to prostate cancer is warranted.

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KEY POINTS

QUESTION: Is ^{67}Cu -CuSarbPSMA therapeutically efficacious.

PERTINENT FINDINGS: ^{67}Cu -CuSarbPSMA appears as efficacious as an agent already used in clinical practice.

IMPLICATIONS FOR PATIENT CARE: Theoretical advantages of the $^{64/67}\text{Cu}$ -CuSarbPSMA theranostic pair are the ability to use a chemically-identical radiopharmaceutical for treatment selection, dosimetry and therapy, while the shorter half-life of copper-67 than lutetium-177 may allow closer cycles.

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Figure 1. The chemical structure of $^{64/67}\text{Cu}$ -CuSarbisPSMA.

Figure 2. A) Inhibition of LNCaP tumor growth following treatment with either ⁶⁷Cu-CuSarbPSMA or ¹⁷⁷Lu-LuPSMA I&T, expressed as mean tumor volume ($\pm$ SEM) (n = 5). B) Kaplan-Meier curve of percent survival data, the endpoint represents the day on which tumor size ≥ 1200 mm³ or censoring on day 82.

Figure 3. A) The antitumor efficacy of ⁶⁷Cu-CuSARbisPSMA against LNCaP tumor xenografts, expressed as average tumor size ($\pm$ SEM) ($n = 8$). B) Kaplan-Meier curve of percent survival data, the endpoint represents the point where tumor size ≥ 1200 mm³ or censoring at day 85.

Table 1. Percentage tumor growth inhibition (%TGI) of LNCaP tumors comparing ^{67}Cu -CuSarbPSMA and ^{177}Lu -LuPSMA I&T to a vehicle control.

Group	%TGI^a	P^b
^{67}Cu -CuSarbPSMA (5 MBq)	58	0.017
^{177}Lu -LuPSMA I&T (5 MBq)	65	0.007
^{67}Cu -CuSarbPSMA (30 MBq)	109	<0.0001
^{177}Lu -LuPSMA I&T (30 MBq)	107	<0.0001

^aTGI analysis performed on day 17.

^bP-values are calculated relative to the vehicle control cohort.

Table 2. Percentage tumor growth inhibition of LNCaP tumors treated with ^{67}Cu -CuSarbisPSMA as compared to vehicle.

Group	%TGI^a	P^b
7.5 MBq ^{67}Cu -CuSarbisPSMA	100	<0.001
15 MBq ^{67}Cu -CuSarbisPSMA	112	<0.001
30 MBq ^{67}Cu -CuSarbisPSMA	119	<0.001

^aAnalysis performed on day 13.

^bP-values are calculated relative to vehicle control.