



Selective modification of fluciclovine (^{18}F) transport in prostate carcinoma xenografts

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Received: 24 November 2017 / Accepted: 5 June 2018 / Published online: 15 June 2018
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Abstract

We investigated if previously demonstrated inhibition of fluciclovine (^{18}F) in vitro could be replicated in a PC3-Luc xenograft mouse model. Following intratumoral injection of 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH), alpha-(methylamino)isobutyric acid (MeAIB) or saline, fluciclovine PET tumor-to-background activity was 43.6 (± 5.4)% and 25.3 (± 5.2)% lower in BCH ($n=6$) and MeAIB ($n=5$) injected PC3 Luc xenografts, respectively, compared to saline-injected controls ($n=2$). Partial inhibition of fluciclovine uptake by BCH and MeAIB can be demonstrated in vivo similar to previous in vitro modeling.

Keywords Fluciclovine · Prostate cancer · PC3 · Amino acid transport · Axumin

Introduction

Anti-1-amino-3- ^{18}F -fluorocyclobutane-1-carboxylic acid [FACBC or fluciclovine (^{18}F)] is a newly FDA approved synthetic amino acid analogue PET radiotracer (Axumin[®]) that has demonstrated utility for the detection of recurrent prostate cancer (Schuster et al. 2014b, 2016a, b; Odewole et al. 2016). Similar to glutamine, fluciclovine (^{18}F) (fluciclovine) is believed to be transported primarily via ASCT2,

with lesser contributions by LAT1, SNAT2, and possibly LAT3 (Oka et al. 2012, 2014; Ono et al. 2015; Okudaira et al. 2011, 2013, 2014). The studies establishing transport mechanisms of fluciclovine involved in vitro experiments utilizing competitive inhibition of carbon-14 labeled FACBC uptake in prostate cancer cell lines using synthetic and natural amino acids (Oka et al. 2012; Okudaira et al. 2011, 2013). We believed it would be valuable to determine if similar selective uptake inhibition could be replicated in a more complex in vivo animal model using fluciclovine (^{18}F).

Handling Editor: E. Closs.

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Materials and methods

PC3-Luc cell culture

PC3 human prostate cancer cells (Augusta University, Augusta, GA, USA) expressing Luciferase (PC3-Luc) were maintained in RPMI-1640 medium supplemented with 10% FBS (Atlanta Biologicals, Lawrenceville, GA, USA), 2 mmol/L L-glutamine, 50 U/mL penicillin—50 mg/mL streptomycin, and 500 ng/mL G-418 selective antibiotic. Cells were cultured in a 37 °C incubator with humidified atmosphere of 5% CO₂.

Establishment of human tumor xenografts in mice and intratumoral injection of substrates

After approval from the Institutional Animal Care and Use Committee, eighteen (18) 6-week-old male outbred homozygous nude mice (J:Nu) were purchased from Jackson Laboratory (Sacramento, CA, USA). Using a cold syringe and 27-gauge needle, 1×10^6 PC3-Luc cells in a total volume of 0.1 mL serum-free medium containing 50% matrigel (BD Biosciences, Palo Alto, CA, USA) were injected subcutaneously in the flank under isoflurane anesthesia. Mice were monitored for about 4 weeks till tumor grew to approximately 5 mm by caliper measurement. Intratumoral inhibitor injections of approximately 5 mM (0.2 mL) of BCH, MeAIB or saline (control) were then completed in the three groups of six mice each. All applicable institutional and/or national guidelines for the care and use of animals were followed in this process.

Fluciclovine micro-PET/CT imaging and analysis

60 mins post-intratumoral injection of amino acid transport inhibitors, approximately 4.7 ± 0.4 MBq of fluciclovine was injected into the tail vein of each mouse and each mouse underwent 60-min dynamic micro-PET/CT imaging (Inveon, Siemens Medical Solutions USA, Inc.) under continuous isoflurane anesthesia. Images were reconstructed and thereafter transferred to Mim 6.6 workstation (MIM Software; Cleveland, OH, USA) for interpretation. Using three-dimensional PET-edge/brush tool, regions of interests were drawn around the tumors carefully excluding the central photopenic matrigel core. Fluciclovine activity parameters were also measured within 10 mm region of interest (ROI) in the liver as a background structure. SUV_{max} tumor to background (T/B) ratio time activity curves were computed using specialized workflows accounting for each mouse's body weight, activity injected and image onset time.

In vivo bioluminescence imaging

Bioluminescence imaging to assess for tumor viability was carried out on an IVIS Spectrum Bioluminescence Imaging System (PerkinElmer) following intraperitoneal injection of 1.5 mg of D-luciferin (PerkinElmer). Measurements were performed when signal reached maximal plateau, with a ROI defined for each tumor, and the average radiance ($p/s/cm^2/sr$) values extracted for quantification. Each mouse was scanned separately in supine position, using high-exposure parameters.

Statistical analysis

Dynamic PET/CT time activity curves were plotted over 60 min for each mouse. The trend of mean \pm standard deviation (SD) of fluciclovine uptake in groups of mice injected with BCH, saline and MeAIB as well as tumors to background (liver) ratios were analyzed. Uptake ratios were also calculated as a percent of control (saline) to quantify the effects of intratumoral injections of BCH and MeAIB on fluciclovine uptake. Statistical analysis was done using Microsoft Excel 2010.

Results

After exclusion of five mice due to either non-viability of tumors on bioluminescence or difficulty with visualization on PET imaging, six BCH-treated, five MeAIB-treated and two saline-treated mice were analyzed. There was no significant difference in tumor size across all groups of mice ($P=0.07$). Details of mice body weight, tumor size, administered dose and bioluminescence are shown in Table 1.

Tumor to background time activity curves

As demonstrated in Fig. 1a, tumor to background activity increased over time in all tumor groups. When compared to saline controls ($n=2$), T/B ratios were most suppressed in prostate cancer xenografts injected with BCH ($n=6$) with less of an effect in those injected with MeAIB ($n=5$). Figure 1b normalizes the activity ratio at each time point to that of saline controls and shows that BCH and MeAIB injections, respectively, led to a $44.8 (\pm 6.9)$ and $26.4 (\pm 7.4)\%$ inhibition of fluciclovine uptake across all 10 time points. Due to small sample size, especially in the control group, test of statistical significance in differences could not be computed.

Discussion

Based on a series of in vitro cell uptake and inhibition studies in prostate cancer cell lines as well as siRNA knock-down experiments, transport of fluciclovine (^{18}F) in prostate cancer cells has been postulated to occur mainly via ASCT2 and less so via LAT1 (with possibly a minor role of LAT3) with some contribution by SNAT2 (Okudaira et al. 2011, 2013, 2014; Ono et al. 2015; Oka et al. 2014). Specifically, we observed a trend that BCH exhibited partial (approximately 20%) inhibition of uptake while MeAIB had

Table 1 Demographics, bioluminescence and fluciclovine uptake in PC3-Luc xenografts injected with BCH, MeAIB or saline

Substrate injected (mouse)	Mouse size (gm)	Tumor size (mm)	Fluciclovine dose injected (μci)	Bioluminescence	
				Total flux (p/s)	Average radiance (p/s/cm ² /sr)
BCH ^a (1)	28	12×9.0	128	5.43E+08	1.38E+07
BCH (2)	27.4	7.6×9.0	131	1.75E+08	4.50E+06
BCH (3)	27.5	4.8×5.2	141	2.47E+07	5.12E+05
BCH (4)	27	5.2×5.7	130	3.15E+07	1.21E+06
BCH (5)	30.3	6.7×6.3	128	2.61E+08	6.36E+06
BCH (6)	30	6.0×6.3	123	1.45E+07	4.83E+05
MeAIB ^b (1)	26.3	5.6×5.0	113	1.15E+08	2.93E+06
MeAIB (2)	26.9	6.1×8.9	110	9.37E+07	2.37E+06
MeAIB (3)	28.2	6.0×5.0	141	7.80E+06	3.27E+05
MeAIB (4)	28.8	6.5×7.2	122	8.62E+07	3.31E+06
MeAIB (5)	30	8.2×7.9	122	2.48E+08	5.66E+06
Saline (1)	25	7.6×6.6	137	1.32E+08	3.35E+06
Saline (2)	26.6	6.6×7.1	130	3.90E+07	1.41E+06

^aBCH-2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid

^bMeAIB-α-(methylamino)isobutyric acid

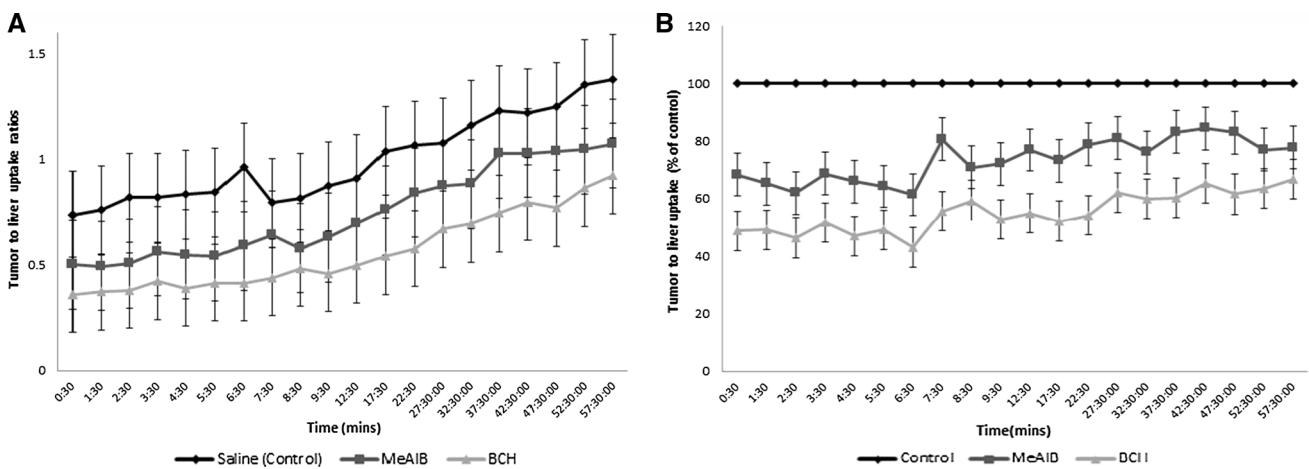


Fig. 1 a Fluciclovine tumor to background ratio time activity curves in PC3 xenografts injected with saline (control), MeAIB and BCH (bars on chart are standard deviation bars). b Fluciclovine tumor to

background uptake in tumors injected with inhibitors normalized as a percent of control (saline) uptake (bars on chart are standard deviation bars)

less (approximately 10%) inhibitory effect compared with controls.

Similarly, in our study, intratumoral injection of BCH, a known LAT1 substrate and system L inhibitor (Kim et al. 2008), resulted in 44% inhibition of *T/B* activity while MeAIB had 25% inhibition compared with controls. Due to differences in methodology between the previous in vitro study and our in vivo study, quantitative results would not be expected to be directly comparable, but overall qualitative results of both studies are similar in that system L inhibitor achieved partial transport inhibition and less so for system A. It is also possible that in the presence of an acidic dense

tumor environment, there was more transport via LAT1 than would be seen from in vitro cell lines alone (Okudaira et al. 2011; Ono et al. 2013; Oka et al. 2012, 2014). The finding of overall agreement between previously published in vitro data and results of our in vivo work adds weight to the translatability of in vitro work on fluciclovine uptake mechanisms.

Our study findings are important because LAT1 expression intensity has been found to correlate with poor patient survival, and could serve as additional prognostic biomarker compared to Ki-67 proliferation index and Gleason score alone (Sakata et al. 2009). As fluciclovine uptake has been described in several tumor types (Schuster et al. 2014a),

delineation of the underlying tumor-specific transport mechanisms, and thus the associated biological processes, is necessary to inform how it can be used to validate and direct the use of current and emerging cancer therapies, particularly those targeting tumor metabolism.

Limitations of this study include small sample size. Also, uneven distribution of inhibitors within the tumors may have occurred, but we believed the use of SUV_{max} for tumor to background ratios, mitigates the effect of this variability. Our ability to directly confirm the contribution of ASCT2 to fluciclovine transport was also limited since highly selective ASCT2 inhibitors were not commercially available at the time of this study. Due to size, fluciclovine uptake in the other background organs could not be easily measured using a 10 mm ROI except in the liver. However, we believed that the liver better reflects biodistribution and is in fact used clinically to aid in interpretation of both fluciclovine and FDG PET-CT (Nye et al. 2007; Schuster et al. 2011). Finally, there could be an imbalance in the magnitude of intravenous fluciclovine reaching the tumor versus directly injected inhibitor; yet, as an initial effort, we sought to determine if there would be any effect even under these extreme conditions.

Conclusion

The results of our in vivo study with PC3-Luc xenografts suggest similar findings compared with earlier in vitro studies: that fluciclovine transport is only partially inhibited by BCH and less so by MeAIB, reinforcing the data suggesting lesser roles for system L (LAT1) and system A (SNAT2) transporters. Further studies with a larger sample size and also utilizing stable shRNA knockdown prostate cancer cell lines with lentivirally encoded shRNA constructs that target specific amino acid transporters are recommended.

Acknowledgements Research reported in this publication was supported in part by the Cancer Animal Models Shared Resource, a core supported by the Winship Cancer Institute of Emory University and Cancer Center Support Grant P30 CA138292.

Funding This study was otherwise internally funded.

Compliance with ethical standards

Conflict of interest Emory University licensed and approved the 18F-fluciclovine patent technology in accordance with the Emory Conflict of Interest Committee policies. M. M. Goodman and Emory University are eligible to receive royalties derived from the sale of 18F-fluciclovine. H. Okudaira and S. Oka are employees of Nihon Medi-Physics Co., Ltd. (NMP) and collaborate with M. M. Goodman and D. M. Schuster on preclinical and clinical studies involving 18F-fluciclovine. F. I. Tade, O. Akin-Akintayo, and D. M. Schuster receive funding/resources from Blue Earth Diagnostics Ltd. and Nihon Medi-Physics Co., Ltd. through the Emory University Office of Sponsored

Projects for research outside that reported in this manuscript. All other co-authors declared no conflicts of interest.

Research involving human participants and/or animals This study was performed with the approval of the Institutional Animal Care and Use Committee. All procedures performed in this study using animals were in accordance with institutional and/or national guidelines and ethical standards for the care and use of animals. No human studies were conducted.

Informed consent No human studies were conducted.

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