Evaluation of a new avidin chase in murine models pre-treated with two ^{111}In labelled biotin analogues

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Abstract

Purpose: the "avidin-biotin system" has been extensively studied for multiple applications to treat solid tumors. In this work, we investigated the effect of a new avidin chase, PWT-Biot1, on the uptake of two different ^{111}In-labelled biotin derivatives in mice limbs intramuscularly pre-injected with avidin.
Methods: two new biotin derivatives (r-BHD and Bis18) were radiolabelled with Indium-111 and injected intravenously in a Balb/c mice (n=48) pre-treated with an intramuscular injection of avidin in the left limb and an inert injection of Matrigel® in the right limb, as negative control. Twelve mice received ^{111}In-r-BHD and twelve received ^{111}In-Bis18. After 30 min, 1 h, 4 h and 24 h, 3 mice per group were anesthetized to acquire in vivo planar images, followed by ex-vivo organ counting. After the synthesis of PWT-Biot1, the same in vivo experiments were performed in two more groups of 12 mice each, but administering the PWT-Biot1 chase, for removing circulating avidin, 10 min before radiolabelled biotin injection.
Results: in each group, we observed high biotin uptake in the left avidinated limb as compared to the right contralateral control limb.
Biodistribution studies showed renal excretion as mainly elimination pathway for both radiopharmaceuticals. The pre-injection of PWT-Biot1 chase increased the uptake in target area of 49% and 35% at 24 h for ^{111}In-r-BHD and ^{111}In-Bis18, respectively.
Furthermore, PWT-Biot1 administration significantly reduced liver activity at 1h and kidney activity at 30 min, 1 h and 4 h after the administration of both biotin derivatives.
Conclusions: our results indicate that PWT-Biot1 chase, by clearing circulating avidin may significantly reduce liver and kidney irradiation and increase the radiolabelled biotin uptake in target avidinated sites.

Key words

Avidin-biotin system; pre-targeting; radiopharmaceutical; imaging; radionuclide therapy.

Introduction

The biotin-(strept)avidin interaction is one of the strongest known non-covalent ligand-protein interaction, with a dissociation constant (Kd) around 10^{-15}M.
Avidin and streptavidin are tetrameric proteins with very similar primary structure and isolated from egg whites and from the bacterium Streptomyces avidinii, respectively. Biotin is a 244-Da water-soluble small molecule, also known as vitamin B7, vitamin H or coenzyme R, founded in biological systems as free or protein-bound biotin in plasma.
Each monomer of avidin/streptavidin is able to bind a single biotin molecule with an extremely rapid and strong binding, remaining unaffected by denaturing conditions, pH, temperature and organic solvents [1].
These exceptional characteristics made this system widely applied in scientific research with several applications, such as immunochemistry, Western blot, ELISA, flow cytometry and protein isolation. In addition to in vitro applications, the researcher explored the possibility to apply this system to in vivo studies.
Radio-immunoimaging (RII) and radio-immunotherapy (RIT) combine the high specificity of monoclonal antibodies (mAbs) to a variety of tumor-cell antigens, with the diagnostic/therapeutic ability of different radionuclides. These techniques have shown promising results in the management of several cancers, but with limited success due to the presence of several limitations. Indeed, the complex pharmacokinetics of mAbs with a prolonged circulation half-life requests long half-life radionuclides to allow tumor imaging, resulting in high radiation dose exposure in patients [2]. In radio-immunotherapy, the accumulation of radiolabelled mAb in non-target organs can result in high systemic toxicity, especially in radiation sensitive tissues such as bone marrow [3].
In this context, a pre-targeting approach can overcome such limitations, reducing the radiation burden to non-target organs and then the systemic toxicity [4]. Pre-targeting based on biotin-(strept)avidin system consists in two main steps: a first injection of "cold" mAb-(strept)avidin complex, with a blood circulation and tumor targeting determined by the mAb pharmacokinetics; a second injection, after a specific time interval, with radiolabelled biotin, with faster pharmacokinetics, directed to the pre-localized mAb-(strept)avidin complex. The time interval allows the elimination of mAbs in blood circulation and healthy tissues, and the optimal accumulation in target site without radiation exposure [5,6].
In breast cancer, the avidin –biotin system has been applied with the so called Intra-operative Avidination for Radionuclide Therapy (IART) technique. The IART approach is a radionuclide treatment able to deliver, one day after surgery, a 20- 25 Gy radiation dose in women who underwent breast conservative surgery [7,8].
One of the potential side effect of this technique is the presence of circulating avidin that binds radiolabelled biotin, thus reducing its accumulation in target areas and increasing the radiation burden to major organs (i.e. bone marrow, kidneys).
To increase tumor-to-non-tumor ratio (T/NT), many approaches have been investigated, such as the use of "clearing agent" to remove circulating avidin that remains in circulation or detaches from the target site. Administration of an avidin chase before the radiolabelled biotin injection, can be an appropriate strategy to reduce the circulating avidin and increase the tumor localization of radiolabelled biotin [9].

This approach could significantly improve avidin-biotin based radionuclide therapy [10]. In this study, avidin has been injected in the left thigh of healthy mice, mimicking the "avidination" of cancer region in IART, providing a target for the radiolabelled biotin subsequently injected. In particular, we investigated the contribution of a new avidin chase (PWT-Biot1) on the target uptake of two different intravenously (i.v.) injected ^{111}In-labelled biotin analogues (r-BHD and Bis18).

Materials and methods

Synthesis procedure of PWT-Biot1

BioUltra lyophilized Avidin from egg white (100 mg) was purchased from Sigma-Aldrich, Italy. The new chase PWT-Biot1 was synthetized as shown in Figure 1 following previously published chemical strategy [11].

Briefly, biotinylated pseudopeptide fragment Biot-Ser-O_2O_c-Lys-Cys-NH_2 was synthesized by standard solid phase peptide synthesis protocol. The biotin moiety was anchored in the N-terminal part of a pseudopeptide sequence designed with the aim to optimize chemical features in terms of biotinidase stability (Ser to form the amide bond with biotin), flexibility (O_2O_c) and solubility (both O_2O_c and Lys as well as Ser) [12]. The thiol moiety of the Cys side chain in C-terminal was employed in the thiol Michael reaction to anchor the peptide to the PWT2 core as the last step of PWT-Biot1 synthesis.

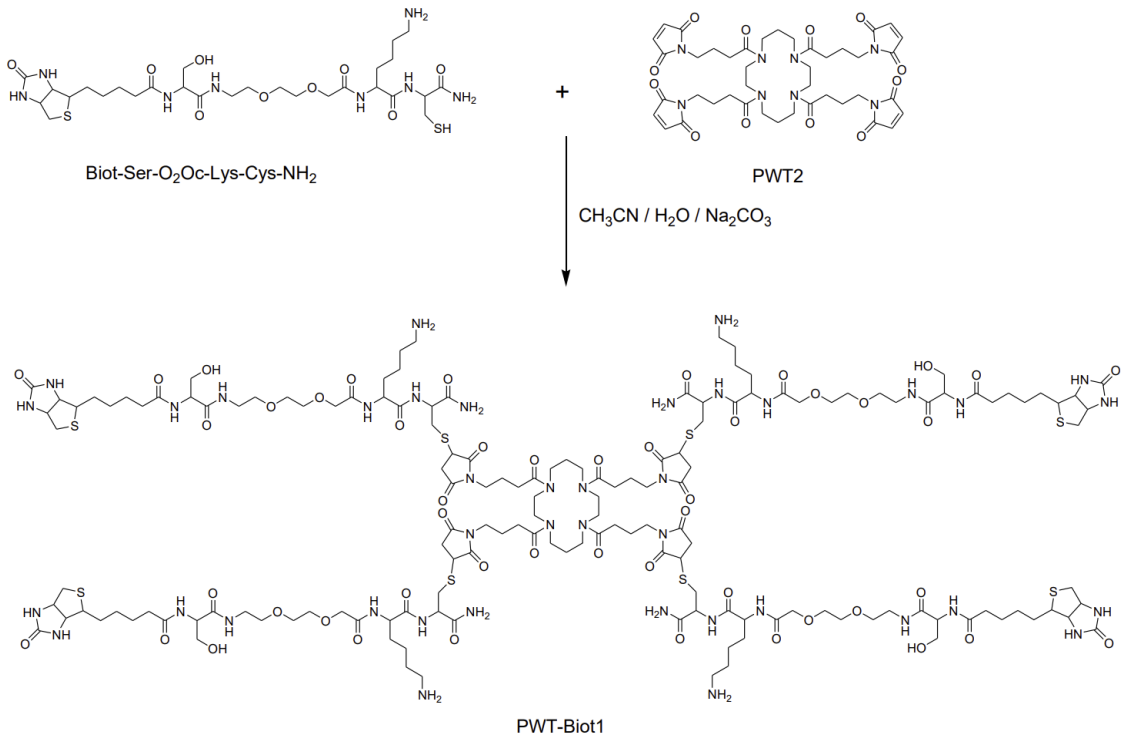

Figure 1. Chemical strategy applied for the synthesis of PWT-Biot1

PWT2 core (6.4 mg; 0.0075 Mmol) was added to a solution of Biot-Ser-O_2O_c-Lys-Cys-NH_2 (22 mg; 0.031 Mmol) in in 2 mL of H_2O:CH_3CN (1:1) under stirring, followed by 50 µL of

Na_2CO_3 5%. The solution was stirred at room temperature for 5 min and directly purified by preparative reversed-phase HPLC using a Water Delta 600 system with a Jupiter column C18 (250 x 30 mm, 300 A, 15 μm spherical particle size). The column was perfused at a flow rate of 20 mL/min with a mobile phase containing solvent A (5%, v/v, acetonitrile in 0.1% TFA), and a linear gradient from 0 to 40% of solvent B (60%, v/v, acetonitrile in 0.1% TFA) over 25 min for the elution of the desired product. Analytical HPLC analyses was performed on a Beckman 126 liquid chromatograph equipped with a Beckman 168 diode array detector.
Analytical purity of PWT-Biot1 was assessed using a Zorbax C18 column (4.6 x 150 mm, 3.5 μm particle size) with the above solvent system (solvents A and B) programmed at a flow rate of 0.7 mL/min using a linear gradient from 0% to 100% B over 25 min. PWT-Biot1 showed ≥ 95% purity when monitored at 220 nm. Molecular weight of the final compound was determined by a mass spectrometer ESI Micromass ZQ.

Radiolabelling of biotin analogues (r-BDH and Bis18) with indium-111 and quality controls
Biotin derivates were synthesized carrying the chelating agent 2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetrazacyclododec-1-yl]acetic acid (DOTA) to perform indirect labelling with metallic radionuclide.
r-BDH was synthetized by labelling one molecule of biotin to one molecule of DOTA. Bis18 was synthesized with two molecules of biotin labelled to one molecule of DOTA.
The labelling procedure was performed as described by Sabatino *et al*, and Pratesi *et al* [13,14]. Briefly, r-BHD and Bis18 were diluted in MilliQ water, to a concentration of 2 mg/ml. Indium-111 chloride ($^{111}InCl_3$) in HCl was obtained from Curium, Italy Srl. $^{111}InCl_3$ solution (5.5 MBq) was added to the reaction vial to achieve a specific activity of 2.7 MBq/μmol, then the vial was incubated in a water bath at 95°C for 8 min. After incubation, radiochemical purity was assessed by Silica Gel Instant Thin Layer Chromatography (ITLC-SG, Gelman) in sodium acetate buffer (1 M, pH=5).
Quantitation of both ^{111}In-DOTA-r-BHD and ^{111}In-DOTA-Bis18 (Rf = 0) and free amount of ^{111}In complexed to diethylenetriaminepentaacetic acid (Rf = 1) was carried out using a linear scanner ((Flow-Count, BIOSCAN, Eckert & Ziegler, Wilmington, MA, USA).

In vivo biodistribution and targeting of ^{111}In-DOTA-r-BHD and ^{111}In-DOTA-Bis18
All animal experiments were carried out in compliance with the local ethics committee and in agreement with national rules and EU regulations.
To perform biodistribution and targeting studies of ^{111}In-DOTA-r-BHD or ^{111}In-DOTA-Bis18, 24 Balb/c mice (8 weeks old, obtained from Envigo, Indianopolis, USA) were used.
Each animal received an intramuscular injection of 100 μl of avidin (20 mg/ml) in the left hind limb and Matrigel® (100 μl, BD-Biosciences, Bergen, NJ, USA) in the contralateral right hind limb as control, as reported in Figure 2.
After 10 minutes animals were injected i.v. in the tail vein with radiolabelled ^{111}In-Biotin derivatives (370 kBq in 50 μl).
At different time points (30 min, 1 h, 4 h, 24 h) post-injection (p.i.), three mice per group were anesthetized to perform imaging studies with a super spatial resolution (SSR) gamma-camera.

After acquiring images, mice were sacrificed by cervical dislocation to collect blood, kidneys, liver and the left and right hind limb for ex-vivo counting with an automated gamma-counter (Perkin Elmer). Data were expressed as the percentage of injected dose per gram (%ID/g).

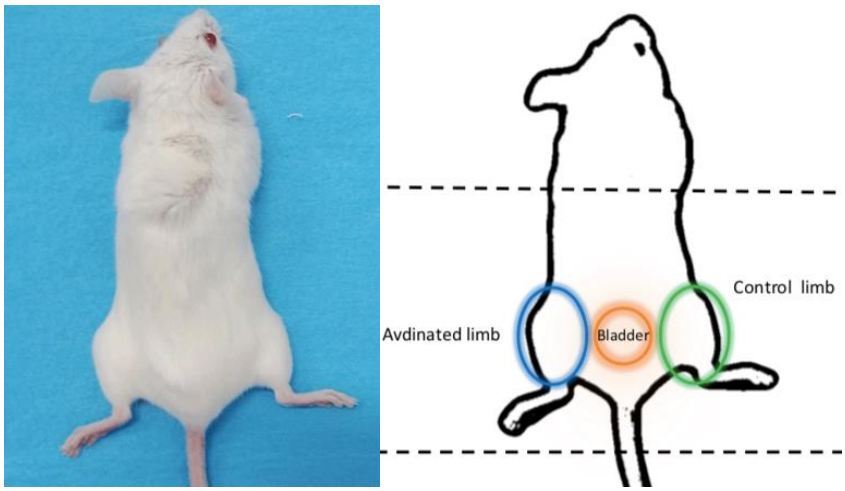

Figure 2: Graphical representation of mouse model for in vivo studies. Mice are injected in the left hind limb with avidin as targeting site, and Matrigel® in the contralateral right hind limb as control.

In vivo evaluation of avidin chase PWT-Biot1

To evaluate the effect of the chase on the uptake of radiolabelled biotin, 24 mice received an additional injection of PWT-Biot1.

Each animal was injected with avidin as described above, followed by chase PWT-Biot1 (20 μg in 50 μl) i.v. administered. After 10 min, radiolabelled biotin was injected i.v. as described above.

At different time points (30 min, 1 h, 4 h, 24 h) p.i., three mice per group were anesthetized to perform imaging studies with a super spatial resolution (SSR) gamma-camera. After acquiring images, mice were sacrificed by cervical dislocation to collect blood, kidneys, liver and the left and right hind limb for ex-vivo counting with an automated gamma-counter (Perkin Elmer). Data were expressed as %ID/g.

Statistical analysis

Statistical analysis was performed using JMP PRO v.15 (SAS Institute, Cary, NC, USA). Normally continuous variables were presented as mean ± SD and non-normally distributed variables as median (max to min). Normality was tested by Shapiro-Wilk test. The differences between (Chase and No-Chase groups) were analysed using t Student or Mann-Whitney tests when appropriate.

Results

Radiolabelling of biotin analogues (r-BDH and Bis18) with indium-111 and quality controls

r-BHD and Bis18 were labelled with ^{111}In with high labelling efficiency (98% and 97% respectively) as revealed by ITLC-SG (Figure 3).

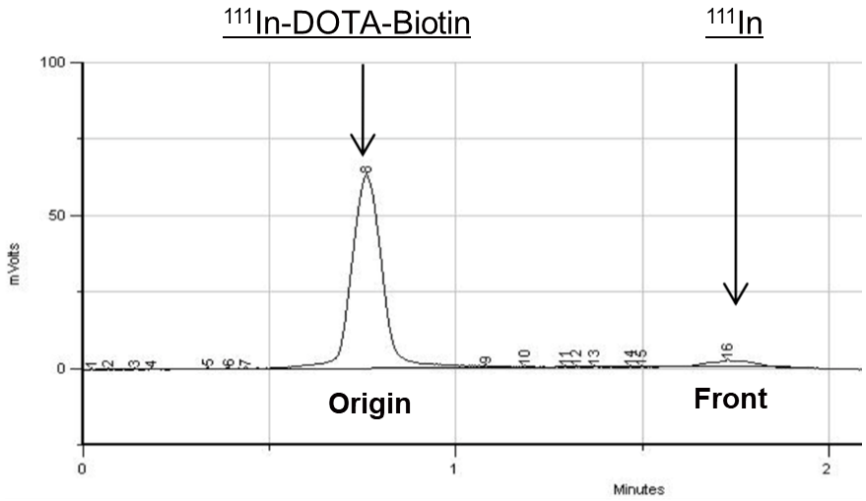

Figure 3. Radiochemical purity was assessed by ITLC-SG. Radiolabelled biotin derivative complex remains at the starting point while free ^{111}In (bound to EDTA) migrates with the solvent front.

In vivo biodistribution and targeting of ^{111}In-DOTA-r-BHD and ^{111}In-DOTA-Bis18

^{111}In-DOTA-r-BHD and ^{111}In-DOTA-Bis18 showed a rapid kidneys uptake, revealed the renal excretion for both the radiopharmaceuticals, with a significant decrease of activity for ^{111}In-DOTA-Bis18 between 4 and 24 p.i. (*p=0.039*) (Figure 4).

The target site (left thigh injected with avidin) exhibited a peak of activity of ^{111}In-DOTA-r-BHD (21.36±2.57 %ID/g) and ^{111}In-DOTA-Bis18 (9.03±0.36 %ID/g) in the first hour p.i., which decreased gradually thereafter, measuring 11.79±4.27 %ID/g and 4.30±1.16 %ID/g, respectively. Negligible uptake has been shown in the contralateral thigh.

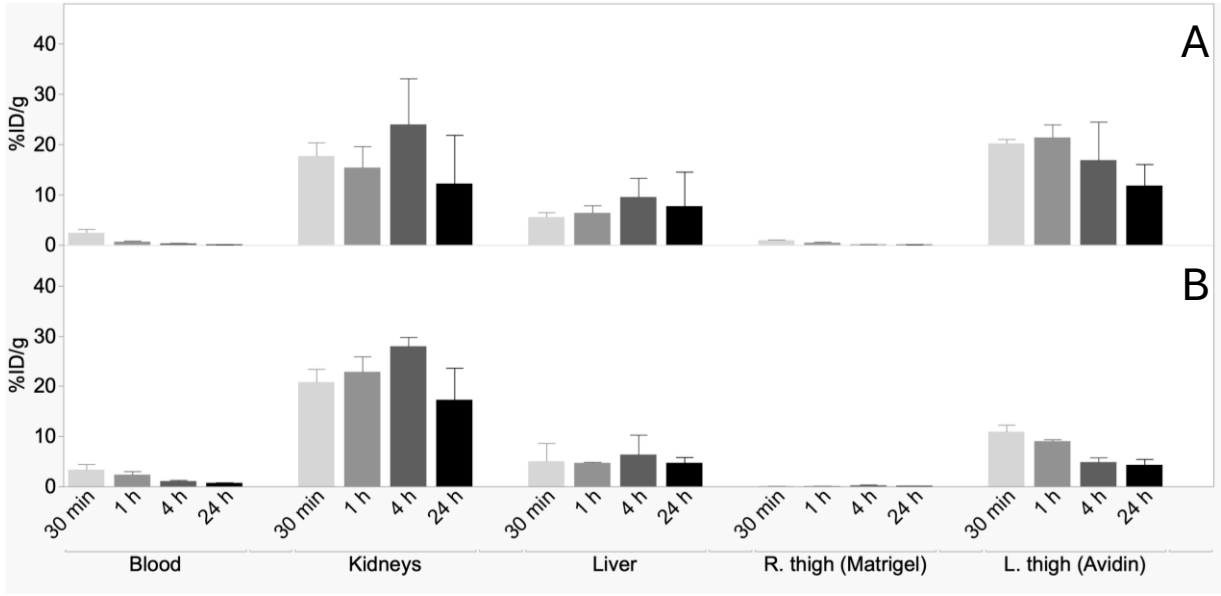

Figure 4. Ex-vivo measurement in tissues and organs (%ID/g), at different time points, of ^{111}In-DOTA-r-BHD (**A**) and ^{111}In-DOTA-Bis18 (**B**), in mice. Post-hoc analysis:

[^{111}In]In-r-BHD

Blood: 30 min *vs.* 1 h, p=0.0025; 30 min *vs.* 4 h, p=0.0001; 30 min *vs.* 24 h, p<0.0001; 1 h *vs.* 24 h, p<0.0001; 4 h *vs.* 24 h, p=0.001.

R. thigh (Matrigel): 30 min *vs.* 4 h, p<0.0001; 30 min *vs.* 24 h, p<0.0001; 1 h *vs.* 4 h, p=0.001; 1 h *vs.* 24 h, p=0.0006.
[^{111}In]In-Bis18
Blood: 30 min *vs.* 4 h, p=0.0005; 30 min *vs.* 24 h, p<0.0001; 1 h *vs.* 4 h, p=0.006; 1 h *vs.* 24 h, p=0.0004
Kidneys: 4 h *vs.* 24 h, p=0.039.
R. thigh (Matrigel): 1 h *vs.* 4 h, p=0.029; 4 h *vs.* 24 h, p=0.01
L. Thigh (Avidin): 30 min *vs.* 4 h, p=0.0006; 30 min *vs.* 24 h, p=0.0002; 1 h *vs.* 4 h, p=0.004; 1 h *vs.* 24 h, p=0.001.

In vivo and ex-vivo evaluation of avidin chase (PWT-Biot1)

In vivo imaging showed a clear and rapid accumulation of ^{111}In-DOTA-r-BHD in target site (left thigh) at 30 min p.i., with a negligible background activity. Ex-vivo studies confirmed the effect of avidin chase on the uptake of biotin on target site (left thigh), which increases of 43% at 30 min p.i. up to 49% at 24h p.i., if compared with left thigh of mice without avidin chase injection (Figure 5 and 6).

The target/blood ratio (%ID/g) confirmed the significant contribution of avidin chase in higher uptake of ^{111}In-DOTA-r-BHD at 4 and 24 p.i. (Figure 7).

In vivo imaging of ^{111}In-DOTA-Bis18 revealed higher background activity, with a less marked activity in target region. However, ex-vivo studies confirmed an increase of activity in target region from 9%, 30 min p.i., to 35 % at 24h p.i., if compared with left thigh of mice without avidin chase injection (Figure 6).

The chase contribution demonstrated also a significant increase in target to blood ratio (%ID/g) at 24 p.i. if compared to mice without avidin injection (Figure 7).

Ex-vivo studies showed as the avidin chase contributed to decrease uptake in non-target organs, such as liver and kidneys for both the radiopharmaceuticals (Figure 6).

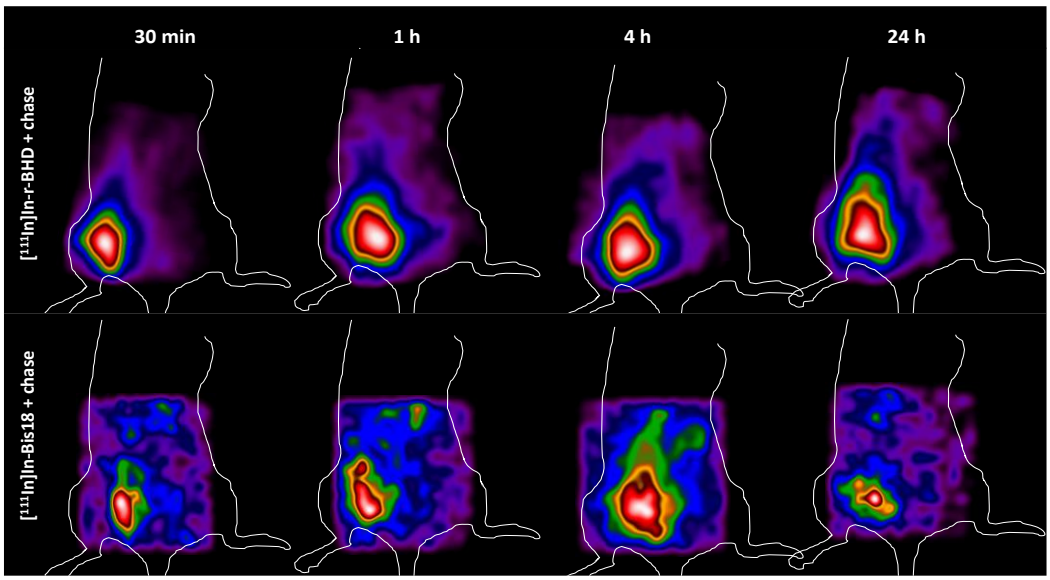

Figure 5. In vivo scintigraphic images of ^{111}In-DOTA-r-BHD ^{111}In-DOTA-Bis18 mice acquired 30 min, 1 h, 4 h, 24 h post injection (p.i.), previously injected with PWT-Biot1.

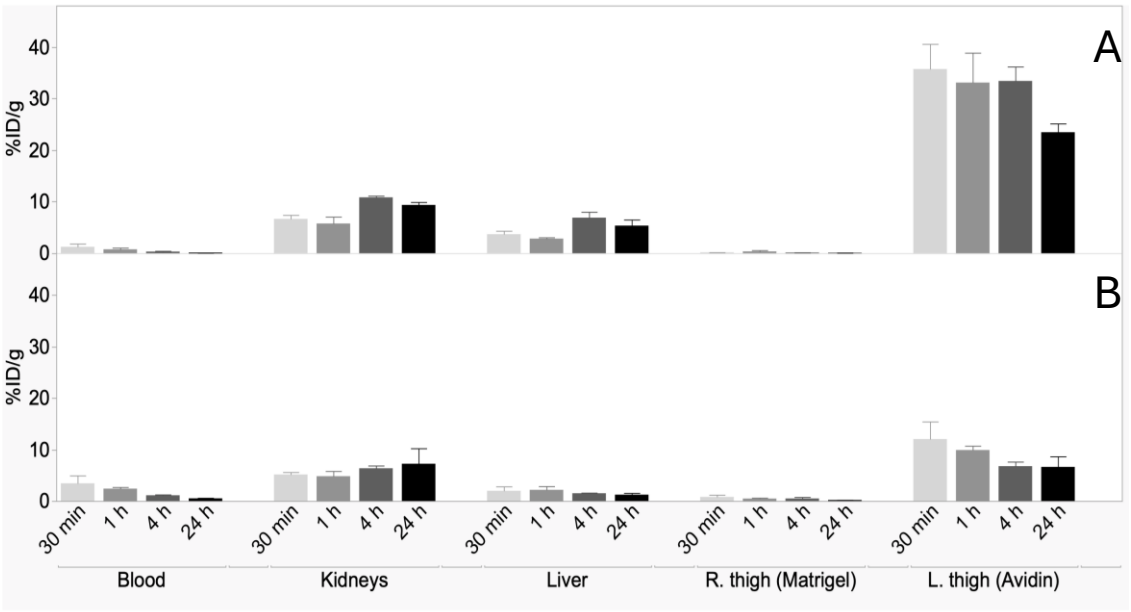

Figure 6. Ex-vivo measurement in tissues and organs (%ID/g), at different time points, of ^{111}In-DOTA-r-BHD (**A**) and ^{111}In-DOTA-Bis18 (**B**), in mice previously injected with PWT-Biot1. Post-hoc analysis:
[^{111}In]In-r-BHD+PWT
Blood: 30 min *vs.* 4 h, p=0.003; 30 min *vs.* 24 h, p<0.0001; 1 h *vs.* 4 h, p=0.038; 1 h *vs.* 24 h, p<0.0001; 4 h *vs.* 24 h, p=0.002.
Kidneys: 30 min *vs.* 4 h, p=0.003; 30 min *vs.* 24 h, p=0.024; 1 h *vs.* 4 h, p=0.0006; 1 h *vs.* 24 h, p=0.003
Liver: 30 min *vs.* 4 h, p=0.002; 30 min *vs.* 24 h, p=0.043; 1 h *vs.* 4 h, p=0.0002; 1 h *vs.* 24 h, p=0.002.
R. thigh (Matrigel): 1 h *vs.* 4 h, p=0.01; 1 h *vs.* 24 h, p=0.002.
L. Thigh (Avidin): 30 min *vs.* 24 h, p=0.004; 1 h *vs.* 24 h, p=0.012; 4 h *vs.* 24 h, p=0.01.
[^{111}In]In-Bis18+PWT
Blood: 30 min *vs.* 4 h, p=0.0009; 30 min *vs.* 24 h, p<0.0001; 1 h *vs.* 4 h, p=0.01; 1 h *vs.* 24 h, p<0.0001; 4 h *vs.* 24 h, p=0.009.
R. thigh (Matrigel): 30 min *vs.* 24 h, p=0.004; 1 h *vs.* 24 h, p=0.04; 4 h *vs.* 24 h, p=0.037.
L. Thigh (Avidin): 30 min *vs.* 4 h, p=0.02; 30 min *vs.* 24 h, p=0.019.

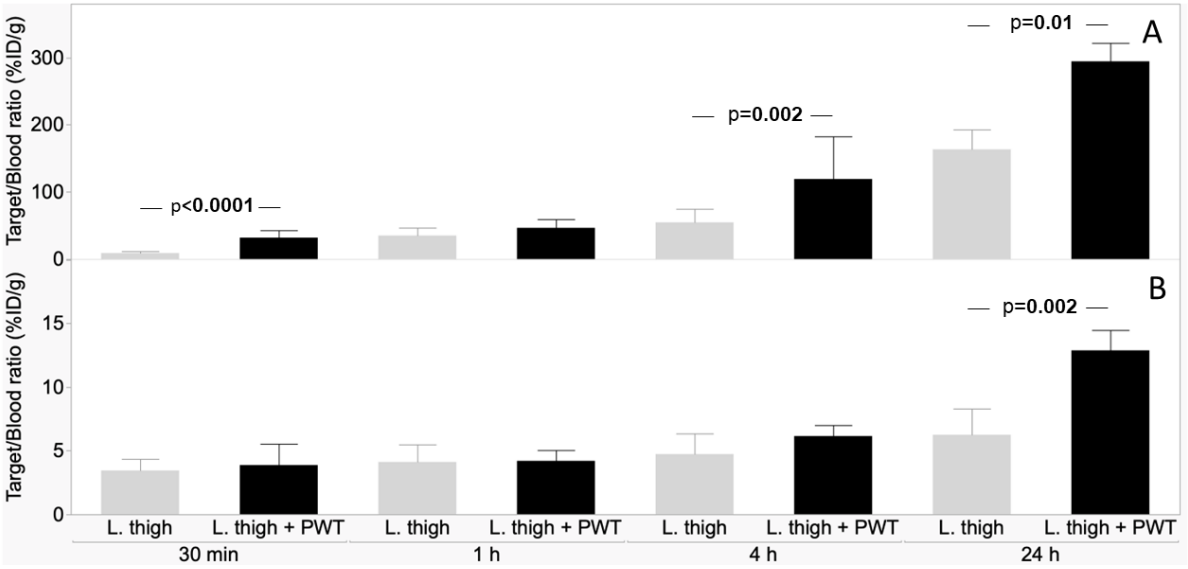

Figure 7. Comparison of target/blood ratio (%ID/g) of left thigh with and without avidin chase injection for both the radiopharmaceuticals, ^{111}In-DOTA-r-BHD (**A**) and ^{111}In-DOTA-Bis18 (**B**).

Discussion

Pre-targeting approach based on the strong avidin-biotin interaction, have shown successful results in clinical oncology, above all in gliomas and breast cancer patients [15].
In this work, we investigated the effect of a new synthetic avidin chase named PWT-Biot1, in association with two biotin analogues radiolabelled with ^{111}In.
The first biotin derivative is a mono-DOTA-conjugated Biotin (r-BHD, Figure 1) and is composed of one Biotin molecule and it has already used in several clinical studies [16]. The second Biotin molecule, called Bis18, is formed by two covalently bound biotin molecules and one DOTA chelating agents and has been described but not used in vivo [14].
Our hypothesis was that PWT-Biot1 would bind and clear circulating avidin reducing its concentration in blood and thus increasing the accumulation of radiolabelled biotin in target areas.
The biodistribution studies of both the radiopharmaceuticals showed a renal excretion as mainly elimination pathway, confirmed by the high kidneys uptake in ex vivo counting. Renal clearance is the preferable route of elimination for radiopharmaceuticals, leading a rapid decreasing of systemic radioactivity and avoiding the liver irradiation, improving safety of patients. At the same time, kidneys are critical organs during IART procedures due to their high absorbed dose and the evaluation of this dose is necessary to avoid a chronic kidney failure.
From biodistribution studies is also clear as the left avidinated region (left thigh) of mice is rapidly targeted by both radiopharmaceuticals, with a negligible uptake in the control region (right thigh).
The data reported from ex vivo studies to evaluate the PWT-Biot1 effect, showed a reduced radioactivity in kidneys and a higher uptake in target region for both the radiopharmaceuticals. This is probably due to the elimination of circulating avidin from PWT-Biot1, resulting in faster accumulation of radiolabelled biotin in target site and less kidneys uptake.
We take note that the ^{111}In-DOTA-Bis18 containing two units of biotin did not increase uptake at levels of the avidinated target leg.
However, standard mono biotin ^{111}In-DOTA-r-BHD already used in humans, confirmed to be an excellent vehicle of radioactivity and therefore we will continue to use it in clinical studies.

Conclusions

Our study confirmed that radiolabelled biotin derivatives can be used to achieve high uptake in avidinated target areas. The use of a "clearing" agent like PWT-Biot1 allowed us to further increase the target-to-background ratio with a great reduction of circulating activity and uptake by non-target organs such as liver and kidneys, reducing their radiation burden.

These results indicate that the use of the PWT-Biot1 in patients undergoing IART or other pre-targeting approaches using the avidin biotin system, could increase the adsorbed dose to the target tissue while lowering radiation dose to blood, kidneys and liver. This is significant especially when these radiopharmaceuticals are labelled with beta- or alfa emitting isotopes for therapeutic purposes.

References

1. Chivers CE, Koner AL, Lowe ED, Howarth M. How the biotin-streptavidin interaction was made even stronger: investigation via crystallography and a chimaeric tetramer. Biochem J. 2011 Apr 1;435(1):55-63. doi: 10.1042/BJ20101593.
2. Nayak TK, Brechbiel MW. Radioimmunoimaging with longer-lived positron-emitting radionuclides: potentials and challenges. Bioconjug Chem. 2009 May 20;20(5):825-41. doi: 10.1021/bc800299f.
3. Loke KS, Padhy AK, Ng DC, Goh AS, Divgi C. Dosimetric considerations in radioimmunotherapy and systemic radionuclide therapies: a review. World J Nucl Med. 2011 Jul;10(2):122-38. doi: 10.4103/1450-1147.89780.
4. Frampas E, Rousseau C, Bodet-Milin C, Barbet J, Chatal JF, Kraeber-Bodéré F. Improvement of radioimmunotherapy using pretargeting. Front Oncol. 2013 Jun 20;3:159. doi: 10.3389/fonc.2013.00159.
5. Li GP, Zhang H, Zhu CM, Zhang J, Jiang XF. Avidin-biotin system pretargeting radioimmunoimaging and radioimmunotherapy and its application in mouse model of human colon carcinoma. World J Gastroenterol. 2005 Oct 28;11(40):6288-94. doi: 10.3748/wjg.v11.i40.6288.
6. Goldenberg, D.M., Chang, CH., Sharkey, R.M. et al. Radioimmunotherapy: Is avidin-biotin pretargeting the preferred choice among pretargeting methods?. Eur J Nucl Med Mol Imaging 30, 777–780 (2003). https://doi.org/10.1007/s00259-002-1089-6.
7. Paganelli G, Ferrari M, Ravasi L, Cremonesi M, De Cicco C, Galimberti V, Sivolapenko G, Luini A, De Santis R, Travaini LL, Fiorenza M, Chinol M, Papi S, Zanna C, Carminati P, Veronesi U. Intraoperative avidination for radionuclide therapy: a prospective new development to accelerate radiotherapy in breast cancer. Clin Cancer Res. 2007 Sep 15;13(18 Pt 2):5646s-5651s. doi: 10.1158/1078-0432.CCR-07-1058. PMID: 17875802.
8. Paganelli G, De Cicco C, Ferrari ME, McVie G, Pagani G, Leonardi MC, Cremonesi M, Ferrari A, Pacifici M, Di Dia A, Botta F, De Santis R, Galimberti V, Luini A, Orecchia R, Veronesi U. IART (Intra-Operative Avidination for Radionuclide Therapy) for accelerated radiotherapy in breast cancer patients. Technical aspects and preliminary results of a phase II study with 90Y-labelled biotin. Ecancermedicalscience. 2010;4:166. doi: 10.3332/ecancer.2010.166. Epub 2010 Nov 1. PMID: 22276027; PMCID: PMC3234029.
9. Li GP, Wang YX, Huang K, Zhang H, Zhang CF. Avidin chase reduces side effects of radioimmunotherapy in nude mice bearing human colon carcinoma. World J Gastroenterol. 2005 Apr 7;11(13):1917-21. doi: 10.3748/wjg.v11.i13.1917.
10. Yudistiro R, Hanaoka H, Katsumata N, Yamaguchi A, Tsushima Y. Bevacizumab Radioimmunotherapy (RIT) with Accelerated Blood Clearance Using the Avidin Chase. Mol Pharm. 2018 Jun 4;15(6):2165-2173. doi: 10.1021/acs.molpharmaceut.8b00027.
11. Guerrini R, Marzola E, Trapella C, Pela' M, Molinari S, Cerlesi MC, Malfacini D, Rizzi A, Salvadori S, Calo' G. A novel and facile synthesis of tetra branched derivatives of nociceptin/orphanin FQ. Bioorg Med Chem. 2014 Jul 15;22(14):3703-12. doi: 10.1016/j.bmc.2014.05.005. Epub 2014 May 13. PMID: 24878361.
12. Wilbur DS, HamLin DK, Chyan MK, Kegley BB, Pathare PM. Biotin reagents for antibody pretargeting. 5. Additional studies of biotin conjugate design to provide biotinidase stability. Bioconjug Chem. 2001 Jul-Aug;12(4):616-23. doi: 10.1021/bc0100096. PMID: 11459467.
13. Sabatino G, Chinol M, Paganelli G, Papi S, Chelli M, Leone G, Papini AM, De Luca A, Ginanneschi M. A new biotin derivative-DOTA conjugate as a candidate for pretargeted

diagnosis and therapy of tumors. J Med Chem. 2003 Jul 3;46(14):3170-3. doi: 10.1021/jm030789z. PMID: 12825956.

14. Pratesi A, Ginanneschi M, Melani F, Chinol M, Carollo A, Paganelli G, Lumini M, Bartoli M, Frediani M, Rosi L, Petrucci G, Messori L, Papini AM. Design and solid phase synthesis of new DOTA conjugated (+)-biotin dimers planned to develop molecular weight-tuned avidin oligomers. Org Biomol Chem. 2015 Apr 7;13(13):3988-4001. doi: 10.1039/c4ob02685c. PMID: 25722026.
15. Grana C, Chinol M, Robertson C, Mazzetta C, Bartolomei M, De Cicco C, Fiorenza M, Gatti M, Caliceti P, Paganelli G. Pretargeted adjuvant radioimmunotherapy with yttrium-90-biotin in malignant glioma patients: a pilot study. Br J Cancer. 2002 Jan 21;86(2):207-12. doi: 10.1038/sj.bjc.6600047. PMID: 11870507; PMCID: PMC2375191.
16. Grana C, Chinol M, Robertson C, Mazzetta C, Bartolomei M, De Cicco C, Fiorenza M, Gatti M, Caliceti P, Paganelli G. Pretargeted adjuvant radioimmunotherapy with yttrium-90-biotin in malignant glioma patients: a pilot study. Br J Cancer. 2002 Jan 21;86(2):207-12. doi: 10.1038/sj.bjc.6600047.