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In vitro and *in vivo* response of PSMA-617 radiolabeled with CA and NCA lutetium-177

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ABSTRACT

The PSMA-targeted radionuclide therapy has been explored since 2015 with radioisotope lutetium-177, whose β^- emission range is adequate for micrometastases treatment. This radioisotope is obtained by two different production routes that directly affect the specific activity of lutetium-177 (non-carrier added and carrier added) and, consequently, the specific activity of radiopharmaceuticals, like ^{177}Lu-PSMA-617. The influence of the specific activity of lutetium-177 on the properties of the radiopharmaceutical PSMA-617 was evaluated through pre-clinical studies. The *in vitro* study pointed to a lower constant of dissociation with non-carrier added lutetium-177 due to the difference in the specific activity. However, competition and internalization assays resulted in similar results for both lutetium-177. Based on these pre-clinical experiments, the total *in vitro* tumor cell binding and tumor uptake *in vivo* were similar, with no influence of the specific activity of the ^{177}Lu-PSMA-617. Regardless the specific activity did not directly affect tumor uptake, the tumor/non-target organs ratios were higher for the radiopharmaceutical labeled with carrier added lutetium-177, which had the lowest specific activity.

1. Introduction

The treatment of cancers through Targeted Radionuclide Therapy (TRT) with radiopharmaceuticals based on small peptides, proteins, and antibodies with high specificity, preserving healthy tissues in the surrounding, has been highlighted in the last decade (Z. S. Morris et al., 2021). Prostate Specific Membrane Antigen (PSMA), discovered in the first decade of 2000, became a critical target to be explored due to overexpression in Prostate Cancer (PCa) and also to be present in approximately 94% of PCa cases (Maresca et al., 2009). These conditions favored the development of PSMA-specific radiopharmaceuticals, particularly with small peptides, such as PSMA-617 radiolabeled with lutetium-177 (Virgolini et al., 2018). The lutetium-177 has a half-life of approximately 6.7 days, emits a β^- radiation with a maximum energy of 497 keV, ideal for treating micrometastases, such as those found in patients non-responsive to the hormonal treatment with Metastatic Castration-Resistant Prostate Cancer (MCRPC) (Mikolajczak et al., 2019). Also, it is possible to evaluate the tumor uptake once the lutetium-177 has a γ-emission of 208 keV that allows the obtaining of scintigraphic images (Mikolajczak et al., 2019).

The potent antagonistic effect of the 2-(Phosphonomethyl)-pentanedioic acid (2-PMPA) on the NAALADase enzyme was determinant to the development of another antagonist, 2-[3-(1,3-dicarboxypropyl)-ureido]-pentanedioic acid (DUPA), which was essential to study the interaction of this molecule with the PSMA receptor (Pomper et al., 2008). The scenario changed in 2009 with the determination of the Glu-Urea-Lys (which binds to the S1′ pocket of the PSMA) as the pharmacophoric group of PSMA antagonist. Additionally, a spacer was added to the molecules to minimize the chelator's steric effect (Maresca et al., 2009). In 2012, a group of researchers determined that the distance

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between the S1′ pocket and the external part of the enzyme site was 20 angstrom (Å) (Chen et al., 2012). In 2013, another research revealed that spacers with 9–11 carbons have better interaction with the enzymatic site of the receptor (Lu et al., 2013). The β-naphthyl group present in the spacer improved the interaction with the hydrophobic pocket at the S1 site of the receptor, which put the PSMA-617, recently developed, in evidence (Benesova et al., 2015).

The first results published in 2015 revealed the potential of ^{177}Lu-PSMA-617 on PCa treatment (Ahmadzadehfar et al., 2015). In the following years, 2016, and 2017, other promising results were obtained with this radiopharmaceutical (Das et al., 2016; Delker et al., 2016; Rahbar et al., 2017; Roll et al., 2017). Furthermore, the most recent publication of the clinical trial entitled "VISION" showed an improvement of 38% on overall survival of the arm treated with ^{177}Lu-PSMA-617 plus gold standard of care (M. J. Morris et al., 2021).

The lutetium-177 is produced in a nuclear reactor by two distinct routes. One of them, the direct production [^{176}Lu(n,γ)^{177}Lu], given by the irradiation of lutetium-176, does not result in 100% radioisotopic conversion. Based on that, there will be non-radioactive lutetium on the sample, the considered carrier added (CA), once the radioisotopic separation is not economically viable (Banerjee et al., 2015). Thus, the purification step might be placed when the radiolabeling of a peptide with CA lutetium-177 without a molar correction (peptide/lutetium-176) results in low radiochemical purity (RCP) (Nanabala et al., 2016). On the other hand, the indirect production route [^{176}Yb(n,γ)^{177}Yb] by irradiating ytterbium-176 and converting to ytterbium-177, which half-life is 1.9-h decays (β^-) to lutetium-177, resulting in non-carrier added (NCA) lutetium-177. In general, the NCA lutetium-177 has higher specific activity (SA) than CA lutetium-177 (Banerjee et al., 2015).

Several scientific studies demonstrate the efficacy of the therapy with ^{177}Lu-PSMA-617, showing significant variability in the SA of the radiopharmaceutical and the lutetium-177 (Ahmadzadehfar et al., 2015; Das et al., 2016; Dash et al., 2016; Hofman et al., 2018; Nanabala et al., 2016). However, many of those works do not report the lutetium-177 supplier, and the SA of the radioisotope and the radiopharmaceutical obtained, in any case. Furthermore, controlled clinical trials with ^{177}Lu-PSMA-617, such as VISION, do not provide information on the SA of the radiopharmaceutical (ICTRP, 2018).

The mass of the PSMA-617 varies in the synthesis procedure, according to the SA of the lutetium-177. In a therapeutic dose of this radiopharmaceutical (200 GBq in a patient with 70 Kg), the amount of the peptide may increase about 100 μg in the case of the ^{177}Lu-PSMA-617 produced with high SA lutetium-177 (NCA), to values of up to 185 μg, depending on the SA of the lutetium-177 with carrier added. Moreover, this excess mass of peptides in circulation could cause a competitive effect by binding to PSMA receptors on tumor cells.

The purpose of this study was to compare two different SA of the radiopharmaceutical ^{177}Lu-PSMA-617 that might lead to a significant variation in the tumor uptake using *in vitro* and *in vivo* models.

2. Materials and methods

2.1. Chemicals

The PSMA-617 was purchased from ABX (Germany). The CA lutetium-177 was obtained from IDB (Holland), with a minimum SA of 500 GBq/mg. The NCA lutetium-177 was purchased from INM (Russia) with a minimum SA of 3000 GBq/mg. Other reagents were obtained with analytical or HPLC grade from Merck (Germany) and Sigma Aldrich (USA). All materials and media for cell culture were purchased from Gibco (USA).

2.2. Radiolabeling of PSMA-617 with ^{177}Lu

The radiolabeling of PSMA-617 with NCA lutetium-177 was performed in a heating block with PSMA-617 (10 μg) diluted in 200 μL of 0.52 M ascorbate buffer pH 4.7 with 740 MBq of $^{177}LuCl_3$ (23 μL) for 30 min at 90 °C.

The radiolabeling of PSMA-617 with CA lutetium-177 (740 MBq) was performed in the same conditions but with a molar ratio of 2.1:1 (PSMA-617/lutetium-176) (18.49 μg of PSMA-617 and 29.75 μL of $^{177}LuCl_3$).

2.3. Radiochemical yield determination

Two chromatographic systems determined the percentage of radiochemical yield (%RCY). Thin Layer Chromatography (TLC-SG) was performed on silica gel 60 plate (Merck, Germany) and 0.1 M citrate buffer pH 5.0. The TLC-SG strips were quantified with a radio TLC-scanner (Scan-RAM, Lablogic, England), in this system, the ^{177}Lu-PSMA-617 showed Rf 0.1–0.2 and the free lutetium-177 Rf 0.9–1.0. Radio-High Pressure Liquid Chromatography (radio-HPLC) (Prominence, Shimadzu, Japan) analysis was conducted using a C-18 column (Waters model Xterra RP 18.5 μm, 4.6 × 150 mm), eluted at a flow rate of 0.4 mL/min by (A) water 0.1% TFA and (B) acetonitrile, in the following gradient mode: 0–2 min 17% B; 2.01–5.99 min 30% B; 6–20 min 17% B. In this system, the lutetium-177 and ^{177}Lu-PSMA-617 were eluted with approximately 4.3 min and 12.17 min, respectively.

2.4. ^{177}Lu-PSMA-617 in vitro assays

The *in vitro* assays were performed with PSMA-617 radiolabeled with NCA and CA lutetium-177. RMPI 1640 medium plus 10% v/v Fetal Bovine Serum (FBS) with 100 UI/mL of penicillin and 300 μg/mL of streptomycin were used to cultivate the LNCaP (Lymph Node Carcinoma of the Prostate) tumor cells. For all *in vitro* assays, 6-well plates were used, with 2×10^5 cells per well. The research group from Fundação Pio XII, Barretos – Brazil (Hospital de Amor) kindly donated LNCaP cells.

2.4.1. Saturation assay

The ^{177}Lu-PSMA-617 (NCA and CA lutetium-177) was diluted with RMPI 1640 to prepare several stock molar concentrations (0.01 nM, 0.05 nM, 0.1 nM, 0.6 nM, 1.5 nM, 2.0 nM, 2.5 nM, and 3.0 nM) and 1 mL of each molar concentration was added per well. The plates were incubated for 1 h at 2–8 °C. Cells were washed (0.1 M PBS pH 7.4, 1 mL, two times), bursted with 2 mL of 1 M sodium hydroxide, and measured in a γ-counter (D5002 Cobra II Packard-Canberra, USA). The concentration of ^{177}Lu-PSMA-617 bonded to the cells was calculated in pMol. This assay was executed in octuplicate, and the K_d was statistically compared.

2.4.2. Competition assay

A stock solution of 5 nM of ^{177}Lu-PSMA-617 (NCA and CA lutetium-177) in Roswell Park Memorial Institute (RPMI) 1640 medium was prepared. It was added 1 mL per well for the total binding and 1 mL of this stock solution (75 nM PSMA-617) as a competitor, added together with the radiolabeled PSMA. The plates were incubated for 1 h at 2–8 °C. Cells were washed (0.1 M PBS pH 7.4, 1 mL, two times), bursted with 2 mL of 1 M sodium hydroxide, and measured in a γ-counter, and the concentration of ^{177}Lu-PSMA-617 bonded to the cells was calculated in pMol. This assay was executed in triplicate, and the specific binding was statistically compared.

2.4.3. Internalization assay

The molar concentration of the ^{177}Lu-PSMA-617 (NCA and CA lutetium-177) used in this assay were based on K_d calculated previously. It was added 1 mL of 1.715 nM of ^{177}Lu-PSMA-617 (CA lutetium-177) and 1 mL of 0.7732 nM of ^{177}Lu-PSMA-617 (NCA lutetium-177). The plates were incubated for 1 h at 37 °C. Then, washed with PBS (1 mL, two times), followed by an incubation step of 5 min at room temperature with glycine buffer (0.05 M glycine, 0.1 M sodium chloride, 1:1 v/v, pH 2.8) to remove all radiopharmaceutical bonded to the cell surface, and

the last step to burst the cells with 2 mL of 1 M sodium hydroxide. The activity was measured in a γ-counter and converted into a percentage of internalization and the results were statistically compared. This experiment was executed in sextuplicate.

2.5. ^{177}Lu-PSMA-617 in vivo studies

For pharmacokinetics and biodistribution studies in a healthy mice model, 7.4 MBq of ^{177}Lu-PSMA-617 were administered through a tail vein in male *BALB/c* mice (3–4 months old). After anesthesia (2% of isoflurane), blood samples of 75 μL (orbital plexus) were collected at 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h for the pharmacokinetic study, and the samples were measured in a γ-counter and converted into the total percentage of injected activity in the blood (% IA total blood), and the blood half-life was calculated. For biodistribution, the mice were sedated (2% of isoflurane) and euthanized (cervical dislocation) at 15 min, 30 min, and 1 h after injection. Lungs, spleen, liver, kidneys, intestines, muscle, and bone were collected and measured in γ-counter and converted into the percentage of injected activity per organ/gram (% IA/g). These assays were executed in quadruplicate.

The tumor-bearing model was injected, subcutaneously, 5×10^6 LNCaP cells in 0.1 M PBS pH 7.4 on the left flank of male *SCID* (*BALB/c* with Severe Combined Immunodeficient) with 3–4 months old (conditions can be found at **supplementary material 1**). After 20 days, 7.4 MBq of ^{177}Lu-PSMA-617 were administrated (tail vein). The mice were anesthetized and euthanized at 30 min, 1 h, 2 h, and 24 h to collect blood, lungs, spleen, liver, kidneys, intestines, muscle, bone, and tumor that were measured in γ-counter and converted to a percentage of injected activity per mL of blood (% IA/mL), % IA total blood, and injected activity per gram (% IA/g). These assays were executed in quadruplicate. For the blocking study, 100 μg of PSMA-617 (diluted in 100 μL of water for injection) were injected intraperitoneally 30 min before radiopharmaceutical administration. After 1 h, the animals were anesthetized and euthanized, and the results were statistically compared with 1 h of no blocking. The animals were supplied from our local animal facility and were handled in compliance with the Guidelines for Care and Use of Research Animals established by the "Comissão de Ética no uso de Animais do Instituto de Pesquisas Energéticas e Nucleares" from Brazil. These experiments were executed in quadruplicate.

The ^{177}Lu-PSMA-617 (NCA and CA lutetium-177) uptake variation in organs that express PSMA (lungs, intestines, and kidneys), liver, spleen, and muscle (non-visceral background representative) was statistically analyzed.

2.6. Statistical analysis

The unpaired *t*-test (two-tailed) with Welch's correction was used for *in vitro* and *in vivo* statistical analysis (GraphPad Prism 8.4.3., GraphPad Software, Inc. San Diego, CA, USA).

3. Results and discussion

3.1. Radiolabeling of PSMA-617 with ^{177}Lu

Radiolabeling of PSMA-617 with NCA and CA lutetium-177 resulted in RCY higher than 95% without purification (Fig. 1). The SA was 74 MBq/μg, and 40.01 MBq/μg for NCA and CA lutetium-177, respectively. The retention time was similar to the cold peptide.

3.1.1. ^{177}Lu-PSMA-617 in vitro assays

The saturation binding experiment showed that ^{177}Lu-PSMA-617 (CA lutetium-177) was bound to the LNCaP cells with a K_d value of 1.65 ± 0.41 nM and B_{max} value of 1123.0 ± 128.7 pmol ($R^2 = 0.9281$). On the other hand, the PSMA-617 radiolabeled with NCA lutetium-177 showed higher affinity to the cells, with a K_d value of 0.77 ± 0.16 nM and B_{max} value of 850.3 ± 53.26 pmol ($R^2 = 0.9180$), once the molecule that shows the smallest dissociation constant represents the one with the most affinity for the receptor (Dong et al., 2015). Although Fig. 2 suggested the data described are similar, the statistical analysis demonstrated a significant difference between K_d ($P = 0.0149$) and B_{max} showed no significant variation ($P = 0.0502$). The difference in the radiolabeling process could explain these results because, for the same amount of radioactivity added to 10 μg of PSMA-617 radiolabeled with NCA lutetium-177, it is necessary to use an excess of approximately 1.8 times of PSMA-617 (that can be presented as non-labeled PSMA-617 or ^{176}Lu-PSMA-617), which can compete for the PSMA receptor and increase the K_d value.

The affinity for the PSMA receptor was determined in a competitive binding assay using LNCaP cells. It was observed an effective blocking of PSMA-617 radiolabeled with NCA lutetium-177 ($P = 0.0003$) and CA lutetium-177 ($P = 0.0020$). No statistical significance was observed compared to total binding ($P = 0.3562$) and specific binding ($P = 0.3603$) for both radiopharmaceuticals (Fig. 3).

Similar binding percentage and internalization results to tumor cells were observed for both radiopharmaceuticals (Table 1).

The PSMA-617 radiolabeled with NCA and CA lutetium-177 demonstrated statistical significance in K_d value on saturation assay, as expected for radiopharmaceuticals with a different SA. According to Hulme and Trevethick (2010), when the specific activities of the ligands are similar, the estimated total concentrations of binding sites should not be significantly different in saturation assays on the same population of receptor binding sites. Based on the radiolabeling conditions used in this work, the SA of both compounds was widely different, which explains the results of the experiment. In the saturation assay, for the same number of cells, the presence of a larger mass of cold peptide, as in PSMA-617 labeled with CA lutetium-177, apparently competed with the labeled peptide for the receptor sites. However, the difference in the SA of the radiopharmaceutical did not affect the specific binding or internalization.

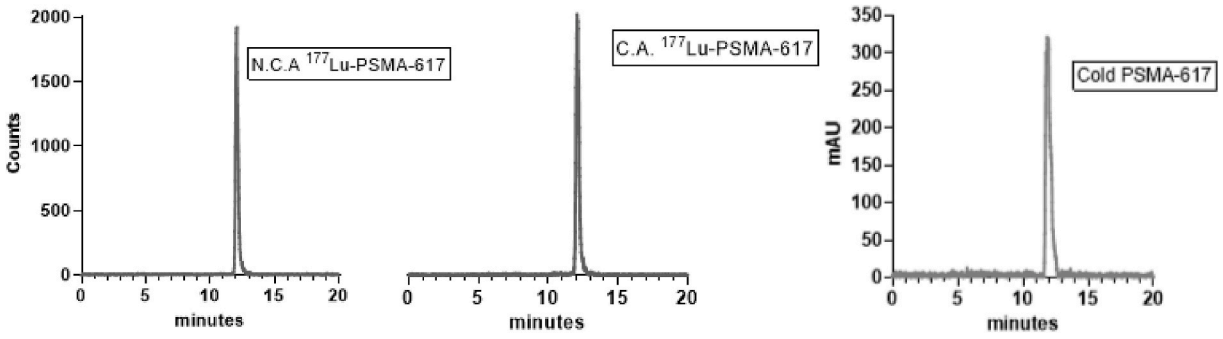

Fig. 1. Radio-chromatographic profile of ^{177}Lu-PSMA-617 (approximately 12.17 min of retention time): NCA e CA lutetium-177, and chromatographic profile of cold PSMA-617 (approximately 11.88 min of retention time).

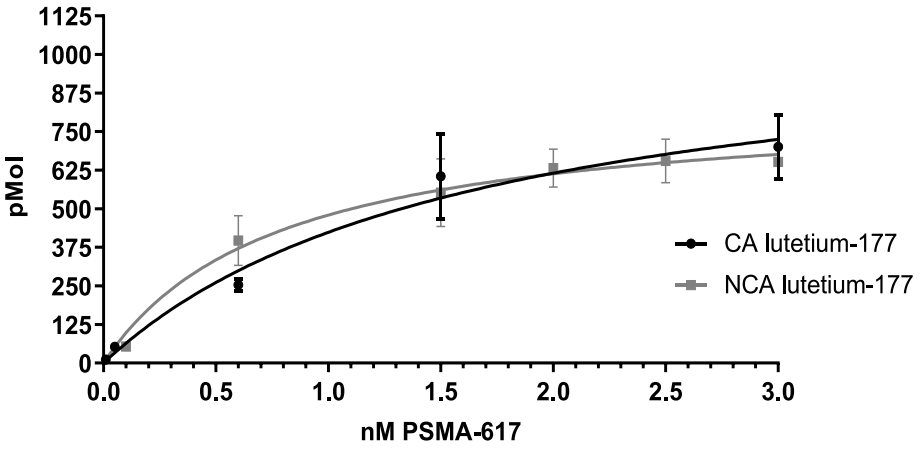

Fig. 2. Saturation curve of PSMA-617 radiolabeled with NCA and CA lutetium-177 (n = 8). Data expressed as mean and Standard Deviation (SD).

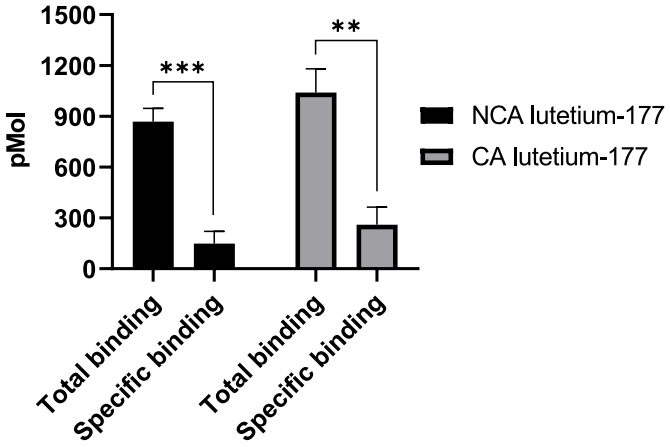

Fig. 3. Cell uptake and specific binding of PSMA-617 radiolabeled with NCA and CA lutetium-177 (n = 3).

Table 1
Internalization of PSMA-617 radiolabeled with NCA and CA lutetium-177 (n = 6).

Lutetium-177	% binding to LNCaP cells	
	Cell surface	Internalized
NCA	69.7% ± 7.9%	30.3% ± 7.9%
CA	72.5% ± 2.5%	27,1% ± 2.5%

3.2. ^{177}Lu-PSMA-617 in vivo assay

The pharmacokinetic experiment revealed a half-life of 13.86 min and 17.74 min for the PSMA-617 radiolabeled with NCA and CA lutetium-177, respectively (Table 2). There was no statistical significance (P = 0.8839) at the clearance parameter, although the clearance of PSMA-617 labeled with NCA 177 lutetium-177 was greater. Rapid elimination of ^{177}Lu-PSMA-617 was observed, and within approximately 2 h, only traces of both radiopharmaceuticals were found in the bloodstream.

Table 2
Pharmacokinetic comparison of PSMA-617 radiolabeled with NCA and CA lutetium-177. Data expressed as best-fit values (n = 4).

Pharmacokinetic parameter	Symbol	Value	
		CA ^{177}Lu	NCA ^{177}Lu
Constant rate of distribution	α	0.31 min	0.35 min
Constant rate of elimination	β	0.039 min	0.049 min
Half-life of distribution	$t_{1/2}$ α	2.21 min	1.99 min
Half-life of elimination	$t_{1/2}$ β	17.74 min	13.86 min
Clearance	CL	0.46 mL min^{-1}	0.74 mL min^{-1}
Volume of distribution	Vd	11.79 mL	15.1 mL

The biodistribution results of ^{177}Lu-PSMA-617 (NCA and CA lutetium-177) (Supplementary Tables 1 and 2) are contemplated in Fig. 4. The lungs uptake of ^{177}Lu-PSMA-617 (CA lutetium-177) was higher at 15, 30 and 60 min when compared to NCA lutetium-177, with a statistical significance after 1 h (0.65 ± 0.07 *versus* 0.18 ± 0.05; P = 0.0008).

The ^{177}Lu-PSMA-617 (CA lutetium-177) also showed a higher retention after 1 h than observed with NCA lutetium-177 on spleen (2.27 ± 0.45% IA/g *versus* 0.39 ± 0.13% IA/g; P = 0.0107), liver (0.16 ± 0.03% IA/g *versus* 0.01 ± 0.01% IA/g; P = 0.0061), intestines (0.18 ± 0.04% IA/g *versus* 0.05 ± 0.02% IA/g; P = 0.0316), and muscle (0.22 ± 0.01% IA/g *versus* 0.04 ± 0.04% IA/g; P = 0.0171). These differences could be related to the slower clearance observed with the peptide radiolabeled with CA lutetium-177, although the difference in pharmacokinetics was not statistically significant for the number of animals studies (N = 4 for each group). Considering the radiolabeling conditions with CA lutetium-177, in a dose of 7.4 MBq, 0.18 nM of PSMA-617 was injected, while in the ^{177}Lu-PSMA-617 (NCA lutetium-177), 0.1 nM of the peptide was administered. This comparative analysis demonstrated that the lower SA that the increased presence of cold peptide in the case of the PSMA-617 labeling with CA lutetium-177 conferred a delay in the elimination of ^{177}Lu-PSMA-617 from visceral and highly vascularized organs such as lungs, liver, and spleen.

The ^{177}Lu-PSMA-617 excretion was predominant renal, with maximum uptake of approximately 64% IA/g at 15 min for both radiopharmaceuticals, without any statistical significance during the whole period studied. It is essential to mention that the renal epithelium has PSMA receptors so that a specific uptake in this organ is expected and the elimination pathway, which makes it a dose-limiting organ (Awang et al., 2018). Although there are few pre-clinical *in vivo* studies with ^{177}Lu-PSMA-617, renal uptake at 1 h was similar to the result found in the literature (approximately 30%AI/g, 1 h after injection) for PSMA-617 radiolabeled with NCA lutetium-177 (ITG, Germany) (Umbricht et al., 2017).

Despite the PSMA-617 radiolabeled with the lower SA having almost the double, in mass, of peptide than with the higher SA, this difference was not sufficient to promote a blocking effect on kidney receptors. Therefore, one could assume a lower renal uptake for the radiopharmaceutical with lower SA, which means higher molarity of cold PSMA-617 molecules in circulation, competing for renal receptors, and exerting a protective effect on the kidneys. However, this fact was not observed. In clinical practice, kidney protectors, such as administering

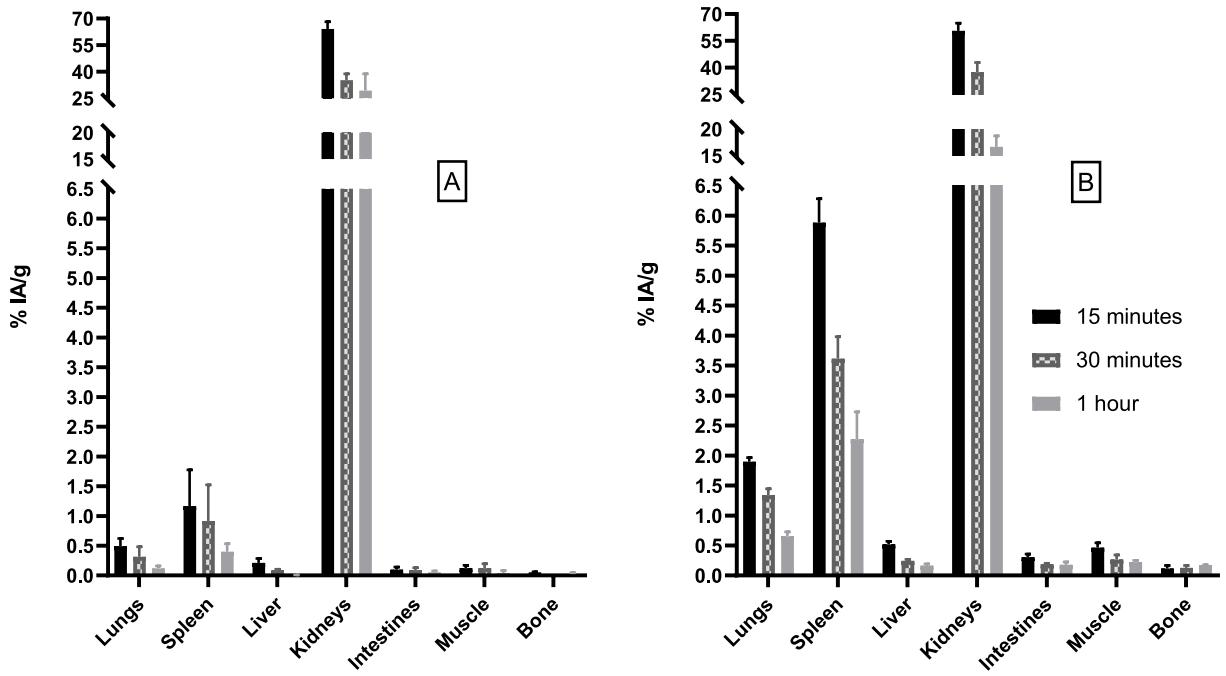

Fig. 4. ^{177}Lu-PSMA-617 biodistribution. [A] represents data of PSMA-617 radiolabeled with NCA lutetium-177 and at [B] with CA lutetium-177 at healthy male mice (*BALB/c*). Results expressed by mean (%I A/g) and SEM (n = 4).

an amino acid solution, is an alternative to reduce the damage caused to the kidneys (Baum et al., 2016).

3.2.1. *Biodistribution in tumor-bearing mice*

The ^{177}Lu-PSMA-617 (NCA and CA lutetium-177) tumor uptake were similar at 30 min (0.4212), 1 h (P = 0.7098), and 2 h (0.1017). After 24 h, only traces of the radiopharmaceutical were detected on the tumor (Table 3).

In contrast to the study with normal animals, the renal uptake of animals with tumors was significantly higher for the group that received radiolabeled PSMA-617 with NCA lutetium-177. In tumor model animals (SCID), the excess mass of cold peptide, in the case of PSMA-617 labeled with CA lutetium-177, appears to have blocked PSMA receptors in the kidneys, promoting renal uptake significantly lower. The amount of peptide administrated in each case, considering the 7.4 MBq administered dose, represents a molar excess of 1.8 for the radiopharmaceutical obtained with CA lutetium-177 (0.1 nM for NCA lutetium-177 and 0.18 nM for CA lutetium-177). This phenomenon was pointed out in a study with ^{111}In-PSMA-I&T, in *BALB/c* nude mice, the authors reported a reduction of 2.5 times on renal uptake with a molar excess of 3.3 times (Chatalic et al., 2016).

After 1 h of injection, rapid blood clearance and significant washout of the organs were observed for both radiopharmaceutical, and tumor uptake showed no significant difference between the two studied groups. Despite that, the ratio of tumor to blood, spleen, liver, kidneys, intestines, and muscle was higher to PSMA-617 labeled with CA lutetium-177 (Table 4), which suggests a better potential for detection of tumors and better contrast in image acquisition, considering the gamma emission of lutetium-177.

Differences between animal species could justify this difference in renal uptake (healthy *versus* tumor-bearing), particularly related to the expression of PSMA receptors in renal tissues. These results point to the importance of studies in human beings that assess the influence of the SA

Table 4
– Comparison of the ratio tumor/blood and tumor/organs (from % IA/g and % IA/mL) after 1 h of ^{177}Lu-PSMA-617 (CA and NCA lutetium-177) administration in tumor-bearing mice.

Ratio	^{177}Lu CA	^{177}Lu NCA
Tumor/Blood	4.35	3.07
Tumor/Lungs	3.79	6.78
Tumor/Spleen	3.89	2.32
Tumor/Liver	8.33	4.34
Tumor/Kidneys	0.04	0.01
Tumor/Intestines	6.59	4.96
Tumor/Muscle	7.79	4.96

Table 3
^{177}Lu-PSMA-617 (NCA and CA lutetium-177) in tumor-bearing mice (male *SCID*) with prostate cancer of LNCaP cells. Results expressed as mean of % IA/g or mL, and SEM (n = 4).

Organ/tissue	^{177}Lu-PSMA-617 (NCA lutetium-177)				^{177}Lu-PSMA-617 (CA lutetium-177)			
	30 min	1 h	2 h	24 h	30 min	1 h	2 h	24 h
Blood	0.90 ± 0.55	0.42 ± 0.09	0.14 ± 0.01	0.01 ± 0.01	0.99 ± 0.08	0.34 ± 0.07	0.30 ± 0.06	0.01 ± 0.01
Lungs	0.38 ± 0.02	0.19 ± 0.01	0.19 ± 0.09	0.02 ± 0.01	0.48 ± 0.16	0.39 ± 0.14	0.30 ± 0.11	0.04 ± 0.01
Spleen	0.97 ± 0.43	0.56 ± 0.09	0.21 ± 0.08	0.01 ± 0.01	0.58 ± 0.03	0.38 ± 0.01	0.28 ± 0.12	0.02 ± 0.01
Liver	0.50 ± 0.08	0.30 ± 0.02	0.14 ± 0.05	0.03 ± 0.01	0.39 ± 0.05	0.18 ± 0.03	0.17 ± 0.06	0.05 ± 0.03
Kidneys	153.3 ± 31.7	150.9 ± 12.9	52.6 ± 24.9	0.75 ± 0.15	60.2 ± 3.94	33.3 ± 6.09	32.4 ± 2.44	0.53 ± 0.42
Intestines	0.36 ± 0.08	0.26 ± 0.03	0.17 ± 0.03	0.01 ± 0.01	0.43 ± 0.04	0.22 ± 0.05	0.17 ± 0.01	0.02 ± 0.01
Muscle	0.51 ± 0.08	0.28 ± 0.05	0.19 ± 0.02	0.01 ± 0.01	0.37 ± 0.05	0.19 ± 0.02	0.05 ± 0.01	0.01 ± 0.01
Bone	0.05 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.01 ± 0.01	0.05 ± 0.01	0.06 ± 0.03	0.05 ± 0.01	0.06 ± 0.04
Tumor	1.59 ± 0.17	1.29 ± 0.22	0.67 ± 0.16	0.04 ± 0.01	1.78 ± 0.12	1.48 ± 0.42	1.10 ± 0.16	0.05 ± 0.15

of the radiopharmaceutical not only on tumor uptake but also on renal uptake.

In this sense, a pre-therapeutic dosimetry study revealed that a single dose of approximately 192 MBq (SA of 44.9 MBq/μg) resulted in 0.88 ± 0.40 Gy/GBq in the kidney (Kabasakal et al., 2015). Another dosimetry investigation with two doses of 6.0 GBq of ^{177}Lu-PSMA-617 (50 MBq/μg) resulted in a kidney-absorbed dose of 0.6 ± 0.2 Gy/GBq, with a maximum cumulative dose of 10.3 Gy (Fendler et al., 2017). Finally, a more recent dosimetric study revealed an absorbed dose of 3.2 ± 0.39 Gy/GBq after a therapeutic dose of 7.4 GBq (100 MBq/μg) (Violet et al., 2019). These dosimetric findings corroborate the renal uptake results found in this present pre-clinical study which demonstrated greater renal uptake for SA of 74 MBq/μg than 40.01 MBq/μg.

A research group in 2011 revealed that non-radiolabeled octreotide coinjected with ^{68}Ga-DOTATATE resulted in a better tumor/liver ratio than patients who did not receive the cold peptide. In that case, the coinjection facilitated the detections of metastatic lesions in the liver (Haug et al., 2011). The tumor/organs ratios obtained to the PSMA-617 radiolabeled with CA lutetium-177 for *SCID* tumor mice model suggest that the cold peptide's excess could enhance the treatment and minimize the kidney dosimetry.

There is no consensus on the SA of the radiopharmaceutical used in the clinic, for example, a lower SA for some cases could result in a competition for the receptor deteriorating the quality of the image. Nevertheless, an excess of the cold peptide can help saturate the non-specific target and reduce toxicity. On the other hand, for agonist radiopharmaceuticals, a non-optimized quantity of mass administrated could induce pharmacological side effects (Luurtsema et al., 2021).

The significant difference in tumor/blood ratio between both radiopharmaceuticals can be correlated to the difference in blood clearance. The pharmacokinetic study of tumor-bearing mice demonstrated a slower blood clearance with the lower SA. The elimination half-life was markedly different between the PSMA-617 radiolabeled with CA and NCA lutetium-177, 91.77 min and 46.81 min, respectively. These pharmacokinetics curves were considered statistically different ($P < 0.0001$) and helped to comprehend the contrast observed at the biodistribution (Fig. 5).

At the blocking experiment, it was observed a reduction on tumor uptake of 97.92% ($P = 0.0265$) and 95.35% ($P = 0.0010$) for PSMA-617 radiolabeled with CA and NCA lutetium-177, respectively, confirming the specificity of binding to PSMA receptors of radiopharmaceuticals obtained from CA and NCA lutetium-177. In addition, renal uptake was also drastically reduced by the presence of the competitor in both groups studied (Table 5).

The results of the biodistribution study in animals with tumors, of ^{177}Lu-PSMA-617 with different specific activities, from the use of CA and NCA lutetium, revealed comparable tumor uptake, suggesting no impact on the therapeutic effectiveness of the radiopharmaceutical. However, as the blood clearance in organs with greater vascularization was slower in the case of CA lutetium, the toxicity for these organs, especially the liver and spleen, might be more significant. On the other hand, renal uptake was significantly lower for the radiopharmaceutical obtained with lower SA, suggesting that the larger mass of peptide present in the formulation may have blocked PSMA uptake in the renal tissue. This blockade could be beneficial as reducing renal uptake could reduce organ dosimetry and related toxic effects. However, this difference in renal uptake was not observed in groups of healthy animals, possibly due to inter-species differences in receptor expression by the kidneys. The findings of this study suggest the importance of investigating the effect of SA of PSMA-specific radiopharmaceuticals in clinical trials.

4. Conclusions

In this work, the effect of two different specific activities of the ^{177}Lu-PSMA-617 was evaluated. In the saturation experiment, the higher specific activity resulted in a lower K_d. Nevertheless, the other *in vitro* experiment, Bmax, internalization, and competition, showed no statistical significance between CA or NCA ^{177}Lu-PSMA-617.

In the *in vivo* experiment with healthy mice, no difference in pharmacokinetic parameters and kidney uptake was not observed. Although, spleen uptake with CA ^{177}Lu-PSMA-617 was significantly higher.

The renal uptake at the tumor-bearing model was statistically lower with CA ^{177}Lu-PSMA-617, suggesting a protective effect on the kidney. It also might lead to higher dosimetry. There was no difference in tumor uptake for both SA studies, but comparison tumor to muscle, liver, spleen, intestines, and kidney was greater with CA ^{177}Lu-PSMA-617.

Therefore, a biodistribution and dosimetric study in humans should be performed to evaluate the impact of CA and NCA lutetium-177 on tumor and non-target organs uptake of PSMA-labeled analogs that are the subject of clinical research.

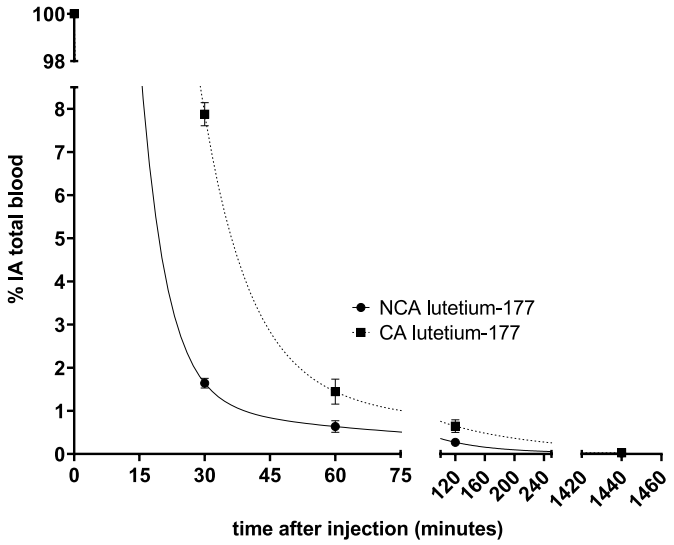

Fig. 5. Comparison of pharmacokinetic curves obtained from a 7.4 MBq ^{177}Lu-PSMA-617 (NCA and CA lutetium-177) tail vein injection in tumor-bearing mice. Results expressed as mean of % IA of total blood and SEM (n = 4).

Table 5
Comparison of blocking and non-blocking biodistribution of ^{177}Lu-PSMA-617 (CA and NCA lutetium-177) at 1 h in the tumor-bearing model. Results expressed as mean of % IA/g and SEM (n = 4).

Organ/tissue	CA ^{177}Lu		NCA ^{177}Lu	
	Non-block	Block	Non-block	Block
Lungs	0.39 ± 0.14	0.29 ± 0.02	0.19 ± 0.01	0.18 ± 0.04
Spleen	0.38 ± 0.01	0.27 ± 0.01	0.56 ± 0.09	0.49 ± 0.01
Liver	0.18 ± 0.03	0.08 ± 0.01	0.30 ± 0.02	0.13 ± 0.01
Kidneys	33.3 ± 6.09	0.32 ± 0.01	150.9 ± 12.9	0.79 ± 0.08
Intestines	0.22 ± 0.05	0.07 ± 0.02	0.26 ± 0.03	0.08 ± 0.03
Muscle	0.19 ± 0.02	0.17 ± 0.03	0.28 ± 0.05	0.17 ± 0.07
Tumor	1.48 ± 0.42	0.03 ± 0.02	1.29 ± 0.22	0.06 ± 0.03

CRediT authorship contribution statement

Cristian Antonio Wieczorek Villas Boas: Writing – original draft, Methodology, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apradiso.2021.110064.

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