THREE NEW 18F-LABELLED PHENYLBENZOTHIAZOLES AS POTENTIAL AMYLOID IMAGING AGENTS

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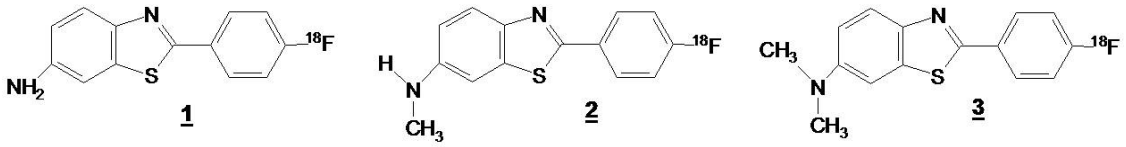
Objectives: Pittsburgh Compound-B (6-hydroxy-2-(4'-N-[^{11}C]methylaminophenyl)-1,3-benzothiazole, PIB) is currently being clinically evaluated for in vivo diagnosis of Alzheimer's disease (AD). Because of the limitations associated with the short half life of carbon-11 we have developed and clinically evaluated fluorine-18 labelled 6-methyl-2-(4'-[^{18}F] fluorophenyl)-1,3-benzothiazole (KS28), which showed high non-specific binding in the human brain. We now developed three new 2-phenylbenzothiazoles, i.e. 6-amino-2-(4'-[^{18}F]fluorophenyl)-1,3-benzothiazole (1), 6-methylamino-2-(4'-[^{18}F] fluorophenyl)-1,3-benzothiazole (2) and 6-dimethylamino-2-(4'-[^{18}F]fluorophenyl)-1,3-benzothiazole (3), which all contain the (methyl)amino-part of the Pittsburgh Compound which is supposed to contribute to specific in vivo binding to amyloid.



Methods: The affinity of the 'cold' compounds for human brain homogenates containing amyloid was determined following a described procedure. Radiolabelling of 3 was realized starting from the nitro-precursor (20 min, 150 °C). Because labeling of 1 and 2 starting from the nitro-precursor failed, we introduced a BOC-protecting group on the amine, which was removed after radiolabelling by heating for 5 min in the presence of diluted hydrochloric acid. Labelling yields ranged between 12 and 41%. The log P value of RP-HPLC purified compounds was determined in octanol/phosphate buffer. Their biodistribution was studied ex vivo at 2 and 60 min p.i. in normal mice and in vivo in a normal rat using a μPET camera. Radiolabelled metabolites in the brain and in blood were determined in a normal mouse at 2, 10, 30 and 60 min p.i.. Brain pharmacokinetics were also studied in a normal monkey performing a μPET study.

Results: Affinity values of the 'cold' compounds (Ki between 3.8 and 10 nM) were in the same range as that of PIB and KS28. The log P value of the monomethyl derivative 2 was 2.4, while the values of 1 and 3 were slightly higher, nl. 3.1 and 3.8, respectively. In normal mice, brain uptake at 2 min p.i. was very high (1: 14.14% ID/g; 2: 12.13% ID/g; 3: 8.84% ID/g) and wash-out from normal brain was rapid (at 60 min p.i.: 1: 0.97% ID/g; 2: 1.45% ID/g; 3: 1.94% ID/g). The μPET studies in a normal rat and monkey confirmed the high brain uptake and wash-out for all compounds. In mouse brain no radiolabelled metabolite was detected for 1. Analysis of mouse brain after intravenous injection of 2 and 3 showed the presence of the demethylated compounds 1 and/or 2 up to 30% 60 min p.i.. Analysis of blood indicated that metabolism of all compounds was slower than that of KS28.

Conclusions: The three compounds show favourable preclinical results. They show a very high brain uptake in normal mice in comparison with PIB (3.60% ID/g) and KS28 (5.65% ID/g) with still a good brain wash-out. A μPET study in a normal rat and monkey confirmed the high brain uptake and wash-out. Further clinical research is planned to evaluate the potential usefulness for in vivo amyloid visualization.

[^{18}F]FBTA2 AS A POTENTIAL RADIOLIGAND FOR IMAGING BRAIN BETA-AMYLOID

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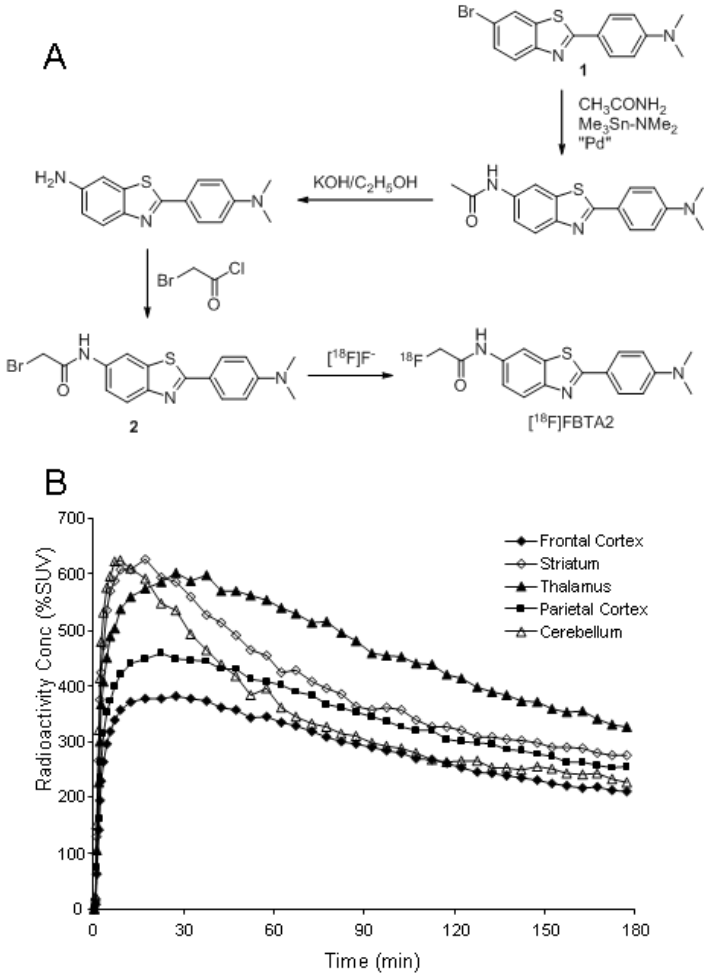
Objectives: [^{11}C]PIB ([methyl-^{11}C]2-(4-(methylamino)phenyl)benzo[d]thiazol-6-ol) is a leading radiotracer for the molecular imaging of A-beta amyloid aggregates in Alzheimer's Disease (AD), and shows rapid and high uptake into brain followed by a quick washout [Mathis C.A. et al. J. Med. Chem., 2003, 46, 2740]. Similarly effective radioligands labeled with longer-lived ^{18}F ($t_{1/2}$ = 109.7 min) are desirable to enable more widespread clinical application. With a view to evaluate more derivatives of PIB, we have exploited an "isoelectronic effect" in the design of PIB analogs [Cai L. et al. J. Med. Chem., 2007, 50, 4746]. These analogs have amido substitutents in place of the hydroxy group. Here we report FBTA2, its labeling with ^{18}F in its fluoroacetamido group, and a kinetic study of this radioligand in monkey with PET.

Methods: Precursor synthesis: The bromoacetamido compound (2) was synthesized in three steps from a known bromo compound (1), itself synthesized in six steps by a known method [Tao X. et al. Chin. J. Chem., 2009, 27, 1] (Scheme). Radiolabeling: [^{18}F]FBTA2 was prepared from 2 with [^{18}F]fluoride ion through an efficient nucleophilic substitution reaction [Briard E. and Pike V.W. J. Label. Compd. Radiopharm., 2004, 47, 217]. Tissue preparation: Human AD brain tissue was homogenized in 500 volumes of phosphate-buffered saline, and aliquots of this suspension (0.100 mL) were used in each tube for in vitro binding assay. PET imaging: A monkey (11.2 kg) was injected with [^{18}F]FBTA2 (6.59 mCi; 3 Ci/μmol). Brain uptake of radioactivity was monitored for 3 h with an HRRT PET camera.

Results: Decay-corrected radiochemical yields of purified and isolated [^{18}F]FBTA2 were about 5%. FBTA2 was found to have high affinity (K_i = 4.6 nM) for AD brain homogenates in vitro (c.f. K_D = 7.2 nM for PIB). The uptake of radioactivity into monkey brain after i.v. injection of [^{18}F]FBTA2 was high (4.00–6.50 SUV at 5–10 min), and more than that of [^{11}C]PIB (2.00–3.00 SUV at 4–6 min). The ratio of radioactivity at its maximum to that at 180 min was 2–3, and comparable to that for [^{11}C]PIB at its maximum compared to that at 60 min [Mathis C.A. et al. J. Med. Chem., 2003, 46, 2740].

Conclusions: FBTA2 showed slightly higher binding affinity than PIB in vitro, and was easily labeled with fluorine-18. The brain kinetics of [^{18}F]FBTA2 has slower washout than that of [^{11}C]PIB in monkeys. Further evaluation of [^{18}F]FBTA2 in AD patients is planned.



Panel A. Synthesis of [^{18}F]FBTA2. Panel B. Time course of radioactivity uptake into brain regions after i.v. injection of [^{18}F]FBTA2 into rhesus monkey.

SYNTHESIS AND EVALUATION OF AROMATIC FLUORINATED [F-18]PIB ANALOGS AS ABETA PLAQUE PET IMAGING AGENTS

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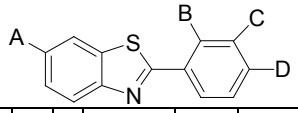
Objectives: We have been interested in the development of PET radioligands for use in non-invasive imaging of Alzheimer's disease (AD). These efforts have led to the development of neutral, lipophilic derivatives of thioflavin-T as potential PET radioligands for the detection and quantification of amyloid-β (Aβ) plaque deposits that are a hallmark of AD. One of these thioflavin-T derivatives, {N-methyl-[C-11]}2-(4'-methylamino-phenyl)-6-hydroxybenzothiazole ([C-11]PIB), has demonstrated the ability to detect Aβ plaque deposits in vivo in humans and has served as our lead compound in this series [1-3].

Methods: We have used PIB as a starting point in our continued efforts to develop [F-18]-labeled analogs suitable for Aβ plaque imaging studies. We have previously reported on several fluoroalkyl analogs[4]. In addition, one aromatic fluoro-substituted analog (2) has previously been identified as a potential lead compound and has been utilized in human subject research. We now report on several additional aromatic fluoro analogs of PiB that have been evaluated using in vitro measures (log P_{C18}, and Ki), as well as ex vivo measures from rodents (normal brain uptake and clearance) to determine their suitability as in vivo PET imaging candidates.



Cmpd	A	B	C	D	Log P_{C18}	Ki (nM) Aβ(1-40)	2 min uptake * (%ID-kg)/g	2/30 min ratio
1	OH	H	H	$NHCH_3$	1.2	4.3[1]	0.21[1]	12[1]
2	OH	H	F	$NHCH_3$	1.7	5.9	0.31	8.4
3	OH	F	H	$NHCH_3$	1.5	9.7	0.20	41
4	$NHCH_3$	F	H	OH	1.5	1.2	0.24	18
5	$NHCH_3$	H	F	OH	1.3	5.3	0.21	29

* Average of triplicate determinations

Results: Several of these compounds (2, 3, and 4) are currently being evaluated as part of FDA-approved IND studies.

Conclusions: Excellent in vitro and ex vivo properties and encouraging non-human primate imaging results have led us to identify compounds 3, 4, and 5 as suitable for further evaluation in in vivo human studies

Research Support: The authors acknowledge research support from NIH (R01 AG18402). GE Healthcare holds a license ageement wih the University of Pittsburgh for the commercial use of the compounds in this abstract and Drs. Klunk and Mathis receive royalty payments for licensed IP.

References: 1. Mathis et al., J. Med. Chem. 46:2740-2754 (2003) 2. Klunk et al., Ann. Neurol. 55:306-319 (2004) 3. Price et al., J. Cereb. Blood Flow Metab. 25:1528-1547 (2005) 4. Mason et al., J. Label. Compd. Radiopharm. 50: S87 (2007)

SYNTHESIS AND EVALUATION OF FLUORINE-18 LABELED BENZOTHIAZOLE ANALOGUES FOR AMYLOID PLAQUE IMAGING PROBE

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Objectives: Benzothiazole (BTA) derivatives are known that have a good in vitro affinity for fibrillar Aβ as well as cross the BBB in brain in sufficient amount to be used as an in vivo probe for visualization. In this study, we have designed three F-18 labeled BTA analogs that F-18 introduced at 6-position instead of OH of [^{11}C]PIB and described the aromatic fluorination of novel F-18 labeled BTA analogues (6-[^{18}F]FBTA-DA (dimethylamine,1), 6-[^{18}F]FBTA-MA (monomethylamine, 2) and 6-[^{18}F]FBTA-A (amine, 3) using iodonium salts as a potential beta-amyloid plaques imaging probe.

Methods: The binding affinities of three fluorine-substituted BTA analogues were performed in fibrillar amyloid b_{1-42}. The aromatic fluorine-18 labeling at 6-position in BTA was carried out under microwave irradiation for 360 seconds using iodonium salt precursors. We performed in the normal ICR mice (7weeks, 25~30g) to evaluate the brain uptake and clearance of three fluorine-18 labeled BTA analogs. After tail vein injection of 6-[^{18}F]BTA analogs, mice were killed at 2, 30 and 60 minute (4~6 mice per point) and the brains were extracted. Radioactivity in tissues was assayed in an automated gamma counter and expressed as percent injected dose per gram normalized to body weight (%ID-kg/g).

Results: Binding affinities (K_i) of 6-F-BTA analogs (6-FBTA-DA= 1.86 nM, 6-FBTA-MA = 7.55 nM, and 6-FBTA-A = 22.24 nM) for aggregated $A\beta_{1-42}$ fibrils were assessed. The aromatic fluorine-18 labeling of BTA analogues were achieved in 20-30% radiochemical yields (decay-corrected) and radiochemical purities were over 95% with >47 GBq/mmol of specific activity. In the (%ID-kg)/g value of 6-[^{18}F]BTA analogs, 6-[^{18}F]BTA-MA showed the high initial accumulation of brain and rapid clearance (0.222 at 2min, 0.042 at 30 min, and 0.024 %ID-kg/g, respectively).

Conclusions: We have developed aromatic fluorine-18 labeling on BTA with sufficient radiochemical yields using iodonium salts. One of 6-[^{18}F]BTA analogs, 6-[^{18}F]BTA-MA showed good binding affinity for fibrillar amyloid b_{1-42} and high initial accumulation and rapid clearance of brain. It is demonstrated that 6-[^{18}F]BTA-MA may be useful for amyloid plaques imaging probe. Now, we are onging to the further PET study in APP/PS Tg mice and wild mice over than 15 month old.

EVALUATION OF [11C]DAA1106 FOR IMAGING AND QUANTIFICATION OF NEUROINFLAMMATION IN A RAT MODEL OF HERPES ENCEPHALITIS

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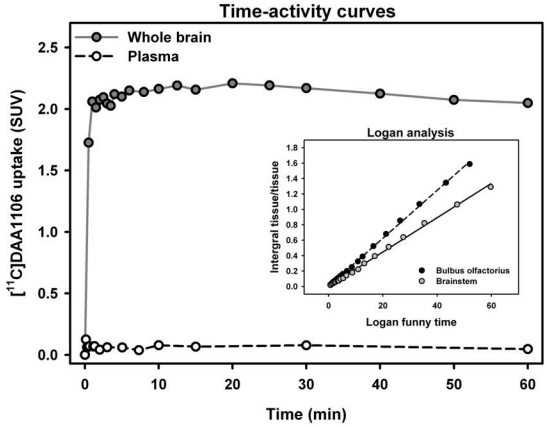
Objectives: Many neurological diseases, like Parkinson's disease and herpes encephalitis, are associated with neuroinflammation. Better understanding of the role of neuroinflammation in these diseases, may direct towards improved treatment. Visualization of neuroinflammation in animal models, using positron emission tomography (PET), can play an important role in this understanding. The peripheral benzodiazepine receptor ligand [^{11}C]PK11195 is most widely used for PET imaging of neuroinflammation, but may not be sensitive enough to visualize mild neuroinflammation. [^{11}C]DAA1106 (N-(2,5-dimethoxybenzyl)-N-(4-fluoro-2-phenoxyphenyl)-acetamide) was developed as a more sensitive tracer [1]. In the present study [^{11}C]DAA1106 was evaluated for PET imaging of neuroinflammation in a rat model of herpes encephalitis.

Methods: Male Wistar rats were intranasally inoculated with the herpes simplex virus type-1 (10^7 PFU in 100μl PBS) or PBS (control). Within a week after inoculation, replicating virus migrated into the brain and induced neuroinflammation. At day 6 or 7 following inoculation the rats received an i.v. injection of [^{11}C]DAA1106 (43±14 MBq). Dynamic PET scans were performed for 1 h, followed by ex vivo biodistribution. Arterial blood sampling was performed to evaluate quantification of tracer uptake.

Results: Brain uptake of [^{11}C]DAA1106 in both infected and control rats was substantially higher than was observed for [^{11}C]PK11195. A significantly higher whole brain uptake of [^{11}C]DAA1106 (31%; p=0.007) was found in infected rats (n=7), when compared to control rats (n=5). The highest increase was found in the bulbus olfactorius (41%; p=0.024), hippocampus (43%; p=0.021) and brainstem (57%; p=0.001). This was consistent with [^{11}C]PK11195 uptake [2], although [^{11}C]DAA1106 also showed significantly increased uptake in other brain areas. Pre-treatment with cold PK11195 (n=4) effectively reduced the uptake of [^{11}C]DAA1106, when compared to control rats (on average 72%; p<0.001). Time-activity curves of plasma and brain showed rapid uptake of [^{11}C]DAA1106 into tissue (figure 1). A high first-pass uptake was found in the lungs (average SUV of 70). The data showed a good fit to the Logan analysis (figure 1; r^2 0.99; p<0.001), with the highest distribution volume in the brainstem (42.9±1.0) and the lowest in the striatum (26.5±0.2), but the very rapid uptake complicated the plasma sampling and kinetic modeling.



Conclusions: [^{11}C]DAA1106 showed a high and specific uptake in the encephalitic rat brain, supporting the use in animal models for neurological diseases. However, quantification of the uptake of [^{11}C]DAA1106 using plasma sampling is not optimal due to rapid tissue uptake, slow tissue clearance and low plasma activity.

Research Support: Stanley Medical Research Institute and DiMI

References: 1 Zhang MR et al. Nucl Med Biol. 30(5): 513-519, 2003 2 Doorduin J et al. Mol Imaging Biol. In press, 2009

CARBON-11 LABELING AND PRELIMINARY EVALUATION OF SSR180575, A HIGHLY PROMISING RADIOLIGAND FOR IMAGING THE PERIPHERAL BENZODIAZEPINE RECEPTOR WITH PET

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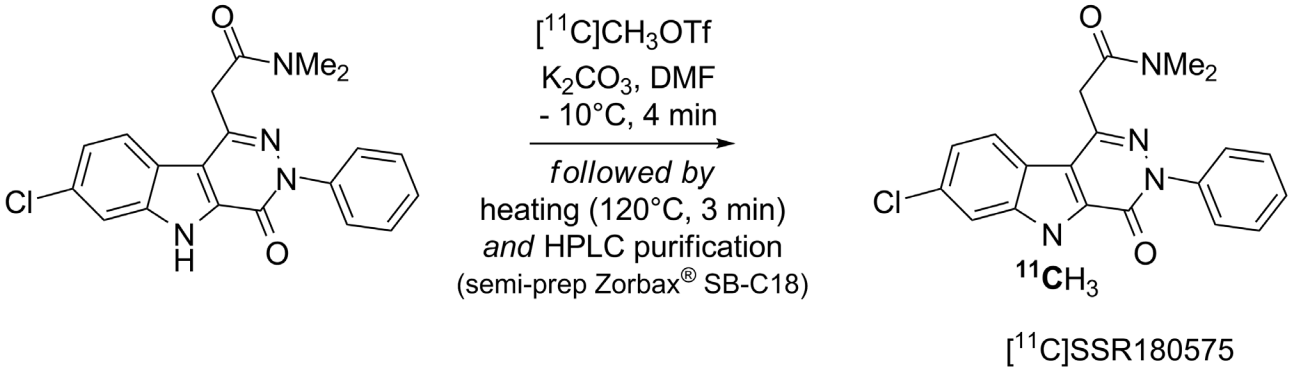
Objectives: Microglia activation is considered as the predominant cellular response to inflammation within the CNS, a process characterized by a drastic change in the morphology of these cells and by the notable overexpression of the peripheral benzodiazepine receptor (PBR or TSPO 18 kDa). Since over two decades, these binding sites are clearly recognised as early markers of neuroinflammation, supporting extensive efforts into the design of radiolabeled ligands for PET imaging [1]. Today, [^{11}C]PK11195 is still considered as the compound of reference, but several new structures, all belonging to other chemical classes (e.g. [^{18}F]FEDAA1106 [2], [^{11}C]PBR-28 [3], [^{18}F]DPA-714 [4] and [^{18}F]DPA-713 [5]) are already proposed as promising alternative ligands. Another attractive chemical class of structures, truly underinvestigated to date, are the indoleacetamides. Within this class, we have labeled SSR180575 [6] with carbon-11, and pharmacologically evaluated it in a rat model of neuroinflammation (unilaterally, AMPA-induced, striatum-lesioned rats) with PET.

Methods: Carbon-11 labeling of SSR180575 (at the N-methylindole function) comprises : (1) trapping at -10°C of [^{11}C]MeOTf in DMF (0.3 mL) containing 0.2-0.3 mg of the indole precursor for labeling and 4 mg of K_2CO_3 (excess) ; (2) heating at 120°C for 3 min ; (3) dilution of the residue with 0.5 mL of the HPLC mobile phase and (4) purification using semi-preparative reversed-phase HPLC (Zorbax® SB-C-18). SSR180575 was also labeled with carbon-11 at its N,N-dimethylacetamide function (step 1 : [^{11}C]MeI, methylacetamide precursor (0.5-1.0 mg), methanolic 1M nBu_4NOH (5 μL), DMF/DMSO (0.1/0.2 mL), -10°C ; step 2-4 : see above). PET-imaging (Focus 220 Concorde) includes control kinetics and displacement experiments with PK11195 and SSR180575 (1 mg/kg).

Results: Starting from a 55 GBq cyclotron-produced [^{11}C]carbon dioxide batch, 4.5-5.0 GBq of [indole-N-methyl-^{11}C] SSR180575 (or [acetamide-N-methyl-^{11}C]SSR180575), > 99% radiochemically pure and ready to inject, were obtained within 25 min. Specific radioactivities ranged from 50 to 90 GBq/μmol. In PET experiments, [indole-N-methyl-^{11}C]SSR180575 showed a higher contrast between the lesioned area and the corresponding area in the intact contralateral hemisphere when compared to [^{11}C]PK11195 (ratio ipsi/contra at 20 min post-injection: [indole-N-methyl-^{11}C]SSR180575 : 2.7, n = 4 ; [^{11}C]PK11195, 1.7, n = 5). Furthermore, [indole-N-methyl-^{11}C]SSR180575 was displaced by PK11195 or SSR180575. Immunohistochemical analyses correlate with PET-imaging and showed strong activation of microglia in and around the lesion.

Conclusions: The decay-corrected overall yields for the preparation of [indole-N-methyl-^{11}C]SSR180575) (or [acetamide-N-methyl-^{11}C]SSR180575) were 19.1-21.2% (n=10). Dynamic μPET studies in rats demonstrate the potential of [indole-N-methyl-^{11}C]SSR180575 to image neuroinflammation.

References: [1] Chauveau et al. Eur. J. Nucl. Med. Mol. Imag. (2008), 35, 2304-2319. [2] Fujimura et al. J. Nucl. Med. (2006), 47, 43-50. [3] Imaizumi et al. Neuroscience Lett. (2007), 411, 200-205. [4] James et al. J. Nucl. Med. (2008), 49, 814-822. [5] James et al. Bioorg. Med. Chem. (2005), 13, 6188-6194. [6] Ferzaz et al. J. Pharmacol. Exp. Ther. (2002), 301, 1067-1078.



IMPROVING TUMOR-TARGETING CAPABILITY AND THERAPEUTICAL EFFICACY OF 111IN/90Y LABELED CYCLIC RGD DIMER WITH PEG4 LINKERS

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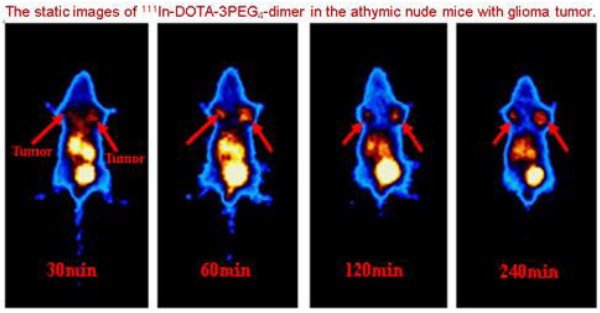
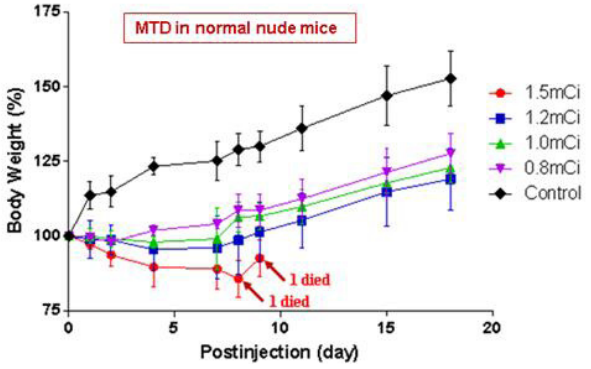
Objectives: Our previous study demonstrated that the addition of PEG_4 linkers between two cyclic RGD motifs in PEG_4-E[PEG_4-c(RGDfK)]$_2$ (3PEG_4-dimer) significantly increased the integrin a_vb_3 binding affinity. In this study, we determine the maximum tolerated dose (MTD) in normal nude mice, and evaluate the therapeutical efficacy of ^{90}Y-DOTA-3PEG_4-dimer for integrin a_vb_3-positive tumors.

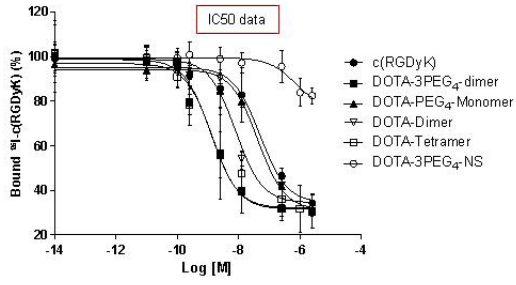
Methods: The nude mice bearing U87MG human glioma xenografts were used for biodistribution, gamma imaging, and therapy studies of ^{111}In/^{90}Y-DOTA-3PEG_4-dimer. The maximum tolerated dose (MTD) in normal nude mice was determined by tail vein injection of escalating ^{90}Y doses (0.8, 1.0, 1.2, and 1.5 mCi). Body weight and health status were determined twice weekly over 18 d. Peripheral blood was collected from the tail vein twice weekly and then tested in a blood analyzer. In the therapy studies, ^{90}Y-DOTA-3PEG_4-dimer was administrated intravenously to mice with U87MG tumors at the doses of 0.3, 0.6 and 1.2 mCi, while using cold DOTA-3PEG_4-dimer and nonsense counterpart as controls.

Results: ^{111}In-DOTA-3PEG_4-dimer exhibited the higher tumor uptake and longer tumor retention than ^{111}In-DOTA-PEG_4-RGD dimer. According to the MTD study, only the 1.5 mCi group experienced animal death on 8 d and 9 d postinjection. The body weight as well as peripheral blood counts displayed an activity-dependant decline, but all values rebounded to baseline by 18 d postinjection. The 1.2 mCi of ^{90}Y-DOTA-3PEG_4-dimer was an appropriate dose for single dose therapy in nude mice according to the MTD study, corresponding to 80.0 mCi/kg, which is much better than 55.5 mCi/kg for ^{90}Y-DOTA-tetramer.

Conclusions: The preliminary experimental data revealed the potential of ^{90}Y-DOTA-3PEG_4-dimer as an agent for the therapy of integrin a_vb_3-positive tumors. The detailed therapeutical study is still in progress.







	c(RGDyK)	DOTA-3PEG$_4$-dimer	DOTA-PEG$_4$-Monomer	DOTA-Dimer	DOTA-Tetramer	DOTA-3PEG$_4$-NS
IC50	4.993e-008	1.250e-009	4.209e-008	8.022e-009	1.372e-009	7.148e-007