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^{68}Ga-NODAGA-RGDyK for $\alpha_v\beta_3$ integrin PET imaging

Preclinical investigation and dosimetry

F. Buchegger[1,2*]; D. Viertl[1]; S. Baechler[3]; V. Dunet[1]; M. Kosinski[3]; C. Poitry-Yamate[4]; C. Rüegg[5]; J. O. Prior[1]

[1]Department of Nuclear Medicine, University Hospital of Lausanne; [2]Department of Nuclear Medicine, University Hospital of Geneva, Switzerland; [3]Institute of Radiation Physics, University Hospital of Lausanne, Switzerland; [4]Laboratory of Functional and Metabolic imaging, Swiss Federal Institute of Technology of Lausanne, Switzerland; [5]Chair of Pathology, Department of Medicine, Faculty of Sciences, University of Fribourg, Switzerland

Keywords

Integrin, $\alpha_v\beta_3$, RGD-peptide, cyclic RGDyK, ^{68}Ga-NODAGA-RGDyK, PET imaging

Summary

Aim: To visualize neovasculature and/or tumour integrin $\alpha_v\beta_3$ we selected the binding moiety Arg-Gly-Asp-D-Tyr-Lys (RGDyK) coupled to NODAGA for labeling with ^{68}Ga. Methods: NODAGA-RGDyK (ABX) was labeled with the ^{68}Ga eluate from the ^{68}Ge generator IGG100 using the processor unit PharmTracer. Biodistribution was measured in female Hsd mice sacrificed 10, 30, 60 and 90 min after i. v. injection of ^{68}Ga-NODAGA-RGDyK for OLINDA dosimetry extrapolated to humans. Tumour targeting was studied in SCID mice bearing A431 and other tumour transplants using microPET and biodistribution measurements. Results: Effective half-life of ^{68}Ga-NODAGA-RGDyK was ~25 min for total body and most organs except liver and spleen that showed stable activity retention. With a bladder voiding interval of 0.5 h the calculated effective dose (ED) was 0.012 and 0.016 mSv/MBq for males and females, respectively. Rapid uptake within 10 min was observed in A431 tumours with dynamic PET followed by a slow release. Biodistribution measurements showed a ^{68}Ga-NODAGA-RGDyK uptake in A431 tumours of 3.4±0.4 and 2.7±0.3%ID/g at 1 and 2 h, respectively. Similar uptakes were observed in a mouse and human breast and ovarian cancer xenografts. Co-injection of excess (5 mg/kg) unlabeled NODAGA-RGDyK with the radiotracer reduced tumour uptake at one hour to 0.23±0.01%ID/g, but similarly decreased uptake in normal organs as well. When unlabeled peptide was injected 15 min after ^{68}Ga-NODAGA-RGDyK, uptake diminished particularly in tumour and adrenals, suggestive of a different binding mode compared with other normal tissues. Conclusion: NODAGA-RGDyK was reliably labeled with ^{68}Ga and revealed a predicted ED of 0.014 mSv/MBq. Tumour uptake was rapid and significant and was chased with unlabeled RGDyK in a similar manner as adrenal uptake.

Correspondence to:
Dr. Franz Buchegger
Department of Nuclear Medicine
University Hospital of Lausanne, CHUV
Rue du Bugnon 46, 1011 Lausanne, Switzerland
Tel. +41/21/314 43 73; Fax: +41/21/314 43 49
E-mail: Franz.Buchegger@chuv.ch

Schlüsselwörter

Integrin, $\alpha_v\beta_3$, RGD-Peptid, zyklisches RGDyK, ^{68}Ga-NODAGA-RGDyK, PET-Bildgebung

Zusammenfassung

Ziel: Zur Darstellung von Integrin $\alpha_v\beta_3$ von Tumoren oder Tumorneugefäßen wählten wir das zyklische Bindungspeptid Arg-Gly-Asp-D-Tyr-Lys (RGDyK) gekoppelt an NODAGA zur Markierung mit ^{68}Ga. Methoden: NODAGA-RGDyK (ABX) wurde mit dem ^{68}Ga-Eluat des ^{68}Ge-Generators IGG100 markiert mit der Prozessoreinheit PharmTracer. Bioverteilungen wurden in weiblichen Hsd-Mäusen 10, 30, 60 und 90 min nach i.v.-Injektion von ^{68}Ga-NODAGA-RGDyK gemessen und als OLINDA-Dosimetrie auf den Menschen projiziert. In SCID-Mäusen, die A431 und andere Tumoren trugen, wurde die Tumoraufnahme mit microPET und Bioverteilungen studiert. Ergebnisse: Die effektive Halbwertszeit von ^{68}Ga-NODAGA-RGDyK war ~25 min im Ganzkörper und den meisten Organen außer Leber und Milz, für die eine stabile Aufnahme gemessen wurde. Die für Frauen und Männer berechnete effektive Dosis (ED) war 0.012 bzw. 0.016 mSv/MBq mit der Annahme eines Blasenleerungsintervalls von 0.5 h. Mit dynamischem PET wurde eine rasche A431-Tumoraufnahme innerhalb von 10 Minuten beobachtet, gefolgt von einem langsamen Abfluss. Bioverteilungen zeigten eine A431-Tumoraufnahme von ^{68}Ga-NODAGA-RGDyK von 3.4±0.4 und 2.7±0.3%ID/g nach 1 bzw. 2 Stunden. Ähnliche Tumoranreicherungen in der Maus wurden in einem Maus- und Humanbrustkrebs gemessen sowie in humanem Ovarialkrebs. Co-Injektion mit einer Überdosis (5 mg/kg) unmarkiertem NODAGA-RGDyK mit dem Radiopharmazeutikum reduzierte die Tumoraufnahme auf 0.23±0.01%ID/g nach 1 Stunde, jedoch wurde eine ähnlich reduzierte Aufnahme auch in andern normalen Organen beobachtet. Wenn unmarkiertes Peptid 15 min nach ^{68}Ga-NODAGA-RGDyK injiziert wurde, verminderte sich die Aufnahme besonders in Tumor und Nebennieren. Diese Beobachtung kann im Vergleich zu anderen Normalgeweben als unterschiedliches Bindungsverhalten gedeutet werden. Schlussfolgerung: NODAGA-RGDyK konnte zuverlässig mit ^{68}Ga markiert werden und zeigte für Menschen eine vorhergesagte ED von 0.014 mSv/MBq. Die Tumoranreicherung war schnell und signifikant. Sie wurde aus Tumor und Nebennieren durch nachgespritztes, unmarkiertes NODAGA-RGDyK in ähnlicher Weise verdrängt.

Integrins are cell surface heterodimers consisting of an α- and β-subunit and 24 different forms have been described (16).

- They mediate cell adhesion, migration and differentiation.
- Integrins are implicated in cancer angiogenesis, invasion and metastasis.
- As transmembrane proteins, they can be expressed in activated and non-activated states and allow cellular outside-in and inside-out signaling (16, 25).
- Viral pathogens may misuse integrins, particularly also $\alpha_v\beta_3$, as receptors for binding to cells and internalisation (24).

The $\alpha_v\beta_3$ integrin is of particular interest for oncology since cilengitide (EMD 121974) is the first integrin inhibitor in phase III clinical development as anti-cancer agent (21).

In difference to quiescent, established vasculature, endothelial cells of tumour neovasculature can express the $\alpha_v\beta_3$ integrin in high amounts. Molecular imaging with positron emission tomography (PET) has been developed on the $\alpha_v\beta_3$ recognition motif arginine-glycine-aspartic acid (RGD) presented as pentacyclic peptide such as RGDfV (cilengitide), RGDyV, RGDfK or RGDyK (10). Radiolabeled RGD peptides have been studied for tumour imaging by different groups. Direct ^{18}F-labeled RGD (1, 2, 15) or chelator conjugated mono- and multimeric peptides labeled with ^{64}Cu (4, 7), ^{68}Ga (11, 12, 14) or ^{111}In (6) have been developed.

Radioconjugates targeting $\alpha_v\beta_3$ integrin have frequently shown, besides high tumour uptake, significant uptake in mouse liver, kidneys and intestines. Co-injection of excess unlabeled peptide together with radiolabeled peptide attenuated uptake of the latter in tumours and in different normal tissues (6, 13, 14).

Currently, it is unclear why RGD pentacyclic radioconjugates are taken up in mouse liver, kidneys and intestines and other normal tissues which normally express lower to undetectable levels of $\alpha_v\beta_3$. The results of co-injection experiments are suggestive of a possible specific targeting of non-tumour tissues in mice. A common but not confirmed hypothesis is that RGD peptides such as those used here recognize, albeit with lower affinity, integrins other than $\alpha_v\beta_3$.

We selected from the literature the RGDyK peptide for coupling with the gallium chelating reagent NODAGA (6, 12) and obtained it in GMP quality for the projected clinical application. We studied here the feasibility of its radiolabeling with ^{68}Ga and performed preclinical evaluations including tumour targeting studies in tumour grafted SCID mice.

Material and methods

NODAGA-RGDyK, (cyclo[L-arginylglycyl-L-alpha-aspartyl-D-tyrosyl -N6-([4,7-bis(carboxymethyl)octahydro-1H-1,4,7-triazonin-1-yl]acetyl])-L-lysyl]), i. e. cyclic Arg-Gly-Asp-D-Tyr-Lys-NODAGA or c(-R-G-D-y-K)-NODAGA was obtained from ABX, Germany that also prepared it in GMP form. Female outbread (CD-1®) Hsd ICR and female CB17 SCID mice were obtained from Harlan Laboratories (Boxmeer, Netherlands). The human cell line A431 [($\alpha_v\beta_3^-$, $\alpha_v\beta_5^+$ (16), epidermoid cancer, ATCC], the mouse mammary tumour 4T1 ($\alpha_v\beta_3^+$) (8) of Balb/c background (kindly provided by Dr F. R. Miller, Michigan Cancer Foundation, Detroit, MI), the human ovarian cancers IGROV-1 [(17), $\alpha_v\beta_3^+$ (18)] and SKOV-3 [$\alpha_v\beta_3^+$ (18), ATCC] and the human breast adenocarcinomas MDA-MB-231 [$\alpha_v\beta_3^+$ (16), ATCC] were cultured at 37°C and 5% CO_2 in RPMI 1640 with glutamax I (Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Chemie Brunschwig AG, Basel, Switzerland), penicillin (50 units/ml) and streptomycin (0.05 mg/ml; Life Technologies Inc., Grand Island, NY, USA). All cells were tested negative for four major potential mycoplasma contaminations (Mycoplasma Detection Kit, F. Hoffmann-La Roche Ltd, Basel, Switzerland) and cultures were renewed every six months after testing.

NODAGA-RGDyK radiolabeling

^{68}Ga was eluted with 0.1 mol/l HCl from the ^{68}Ge generator IGG100 (Eckert & Ziegler, Germany), using the automatic processor unit Modular-Lab PharmTracer (Eckert & Ziegler). NODAGA-RGDyK (20 µg) was radiolabeled with the high activity ^{68}Ga fraction by incubation for 20 min at room temperature. After cartridge purification (part of the automatic processor unit) the ready for use ^{68}Ga-NODAGA-RGDyK was eluted in 50% ethanol through a 0.22 µm sterile filter and diluted into NaCl solution. High pressure liquid chromatography analysis was performed on a µ-Bondapak column (Waters C-18) run with trifluoroacetic acid and acetonitrile.

Dosimetry study

All animal experiments were performed according to the principles of laboratory animal care and national ethical guidelines. The animal experiments have been subjected to authorization and control by the official Canton and Swiss veterinary service on surveillance of animal experiments (VD 2013 et VD 2013.3). Animals were kept under specific pathogen-free conditions, in autoclaved filter-topped cages and bedding in a separate room. Mice had access ad libitum to sterilized food and water. Animals were sacrificed after single experiments.

^{68}Ga-NODAGA-RGDyK (0.5 MBq) was injected i. v. in female, outbread (CD-1®), Hsd ICR mice for biodistribution studies and OLINDA-based dosimetry extrapolation. Mice were sacrificed 10, 30, 60 and 90 min after injection and blood and organs collected, weighed and radioactivity counted compared with a sample of 5% injected activity. Gastrointestinal tissues were collected with their content. Tissue concentrations of activity were then expressed as % of injected activity per gram (% ID/g). Total body activity was determined by addition of all organs and tumour with the carcass that was also sampled.

^{68}Ga-NODAGA-RGDyK biodistribution results were transformed in effective %ID/g and used to calculate single exponential effective half-lives for each organ and the animal. Residence times for each mouse tissue were calculated and maintained for human dosimetry extrapolation without correction for organ masses. Red marrow activity was assumed being equal with blood activity with the same half-life while respecting the respective volumes of

blood and red marrow (18). The urinary bladder model (Mirdose) (23) was used for searching optimal bladder voiding intervals. The urinary elimination fraction of ^{68}Ga-NODAGA-RGDyK was set at 75% with a 40 min biological half-life according the whole-body results. Residence times were introduced into Olinda (22) and radiation doses calculated for female (58 kg) and male (70 kg) human beings.

NODAGA-RGDyK tolerance study

NODAGA-RGDyK was tested at 1000-fold weight excess/kg in five female outbread Hsd ICR mice. No toxicity was expected to occur at this dosage and higher doses were not tested. Mice were weighed from arrival into the lab until tolerance study two months later. Mice with a mean weight of about 35 g were injected 10 µg unlabeled NODAGA-RGDyK (1000-fold excess per kg body weight compared with the maximal projected human dose of 20 µg for a 70 kg patient). Mice were inspected 0.25, 1, 2, 3 and 5 h after injection and then inspected and weighted 2 to 3 times weekly over the next two months. Mice were sacrificed by CO_2 exposure and organs inspected macroscopically by two scientists.

Tumour targeting studies

SCID mice were grafted subcutaneously with human or mouse tumours (1 to 10 x 10^6 cells/animal, depending on the cell line) and biodistribution was studied with tumours having grown to between 0.1 and 0.3 g, generally two to four weeks after cell transplantation. Mice were injected ^{68}Ga-NODAGA-RGDyK i.v. (0.5 MBq/animal corresponding to 0.04 to 0.1 µg peptide). Control mice were injected with radiolabeled peptide together with 5 mg/kg non-radiolabeled NODAGA-RGDyK. Mice were sacrificed at one or two hours after injection, and biodistribution determined by tissue and blood weighting and radioactivity counting against a standard sample of 5% injected activity. Total body activity represents the sum of all activity measured from injected mice (carcass, organs, blood and tumour).

For microPET imaging tail vein cannulated mice were i. v. injected 4 MBq/animal ^{68}Ga-NODAGA-RGDyK. PET was performed on a dedicated small-animal PET scanner (LabPET4; Gamma Medic-Ideas, Sherbrooke, Quebec, Canada). Mice were maintained under 1% isoflurane anesthesia in oxygen at 1 l/min for cannulation and during the entire scanning period; temperature and breathing rate were monitored and maintained constant. 50 min list-mode acquisitions were initiated with the i. v. injection of ^{68}Ga-NODAGA-RGDyK and static imaging recorded 60 to 70 min after injection. An energy window of 250–650 keV and a coincidence timing window of 22.2 ns were used. The list-mode data were sorted into 50 min time frames of 1 min. Images were reconstructed by MLEM iterative method in a cylindrical volume of 46 mm diameters and 3.7 cm length. The voxel was of 0.5×0.5×1.2 mm giving a typical resolution of 1.2 mm at the center of the field of view. The image data were corrected for nonuniformity of the scanner response, dead time count losses, and physical decay to the time of injection.

Chase experiments

With the intention to chase a supposed low affinity binding of ^{68}Ga-NODAGA-RGD in normal tissues like liver, kidneys and intestines, we performed different chase experiments in SCID mice bearing A431 tumours. We injected first [^{68}Ga]-NODAGA-RGDyK i.v., followed 15 minutes later by the i. v. injection of moderate amounts (0.1 to 2.0 mg/kg) NODAGA-RGDyK. Chase was performed mostly as a single, non-labeled NODAGA-RGDyK i. v. injection performed for each mouse 15 min after radiolabel injection. In a few experiments chase was performed in two injections, the first at 15 minutes and the second as described in results. Since it was intended not to reduce tumour uptake, the peptide amount used for chase was reduced as compared with competition experiments. Chase experiments were evaluated with biodistribution studies performed one or two hours after radiopeptide injection.

Results

^{68}Ga-labeling of NODAGA-RGDyK

Labeling of NODAGA-RGDyK with the high activity fraction of ^{68}Ga eluate was efficient and reliable over the period studied. ^{68}Ga-NODAGA-RGDyK showed a single peak on HPLC (▶Fig. 1), contaminants representing <1%. Specific activity in these experiments conducted over one year was between 100 to 250 MBq per 20 µg peptide. This latter amount represents the projected maximal quantity foreseen for injection of patients.

Dosimetry projection for humans

Biodistribution studies were performed after i.v. injection of ^{68}Ga-NODAGA-RGDyK in normal mice (▶Fig. 2) and showed effective half-lives of 25 min for whole body within a range of 18 to 35 min for most normal organs except liver and spleen. Radiopeptide uptake in liver and spleen remained rather constant over the observation period resulting in effective half-lives of 63 and 53 min, respectively. The human effective dose (ED) and effective dose equivalent (EDE) extrapolated from these results was for men 0.012 and 0.015 mSv/MBq, respectively, and for women 0.016 and 0.020 mSv/MBq.

The highest radiation dose was calculated for the urinary bladder wall with 0.139 and 0.188 mGy/MBq (▶Fig. 3a) for men and women, respectively, despite choosing an optimized urinary bladder voiding interval of 30 min. In decreasing order organ contributions to the ED (▶Fig. 3b) of humans would be highest for the urinary bladder wall (58%) followed by liver (10%), stomach (7%), lower large intestinal wall (LLI, 6%), gonads (6%), lungs (4%) and red marrow (3%).

Tolerance towards an excessive dose of NODAGA-RGDyK

The tolerance study showed the absence of toxicity under use of an excessive i. v. overload of animals with peptide. Foreseeing the injection of patients with maximally 20

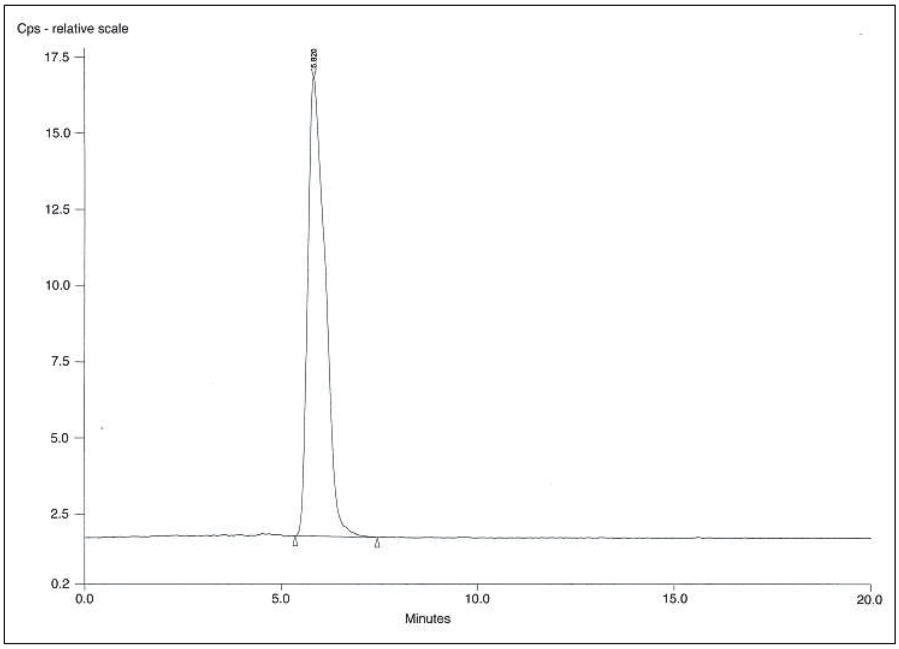


Fig. 1 HPLC profile of ^{68}Ga-NODAGA-RGDyK showing a single peak as obtained in a 20 min labeling of NODAGA-RGDyK at room temperature and cartridge purification that is part of the automatic processing module.

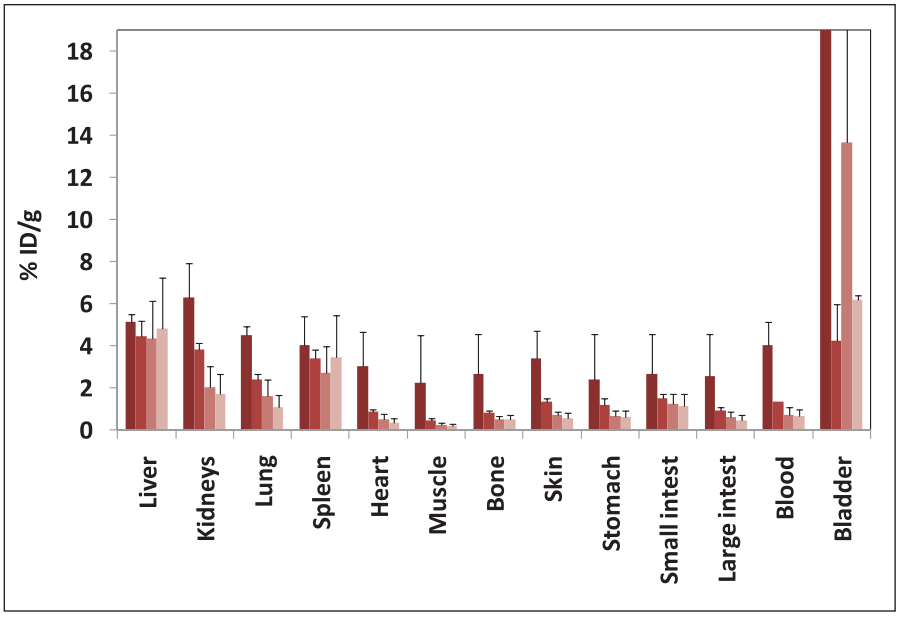


Fig. 2 Biodistribution results corrected for the physical half-life of the isotope are expressed in % of injected dose per gram tissue (%ID/g) 10, 30, 60 and 90 min after i. v. injection of ^{68}Ga-NODAGA-RGDyK in normal mice.

µg NODAGA-RGDyK (depending on labeling efficiency), mice of 35 g were given an i. v. injection of 10 µg unlabeled tracer (1000-fold excess). As shown (▶Fig. 4), the weight curve of the animals observed two months before and two months after injection of peptide remained homogenous without any significant daily or weekly modification. The immediate post injection period of five hours did not reveal any particular behavior of the animals. The macroscopic inspection at necropsy showed a normal organ appearance in all animals.

Tumour localization and biodistribution studies

Tumour uptake in A431 xenografts as measured in biodistribution experiments was 3.4±0.4 and 2.7±0.3%ID/g at 1 and 2 hrs, respectively, indicating good retention of peptide over this period of time. Tumour uptake was reduced to 0.2%ID/g at 1 hour when using co-injection of excess amount unlabeled NODAGA-RGDyK, however, normal tissue activity uptake, except for kidney, was decreases to a similar degree (▶Fig. 5a).

Tumour uptake of ^{68}Ga-NODAGA-RGDyK in different other human cancer xenografts in mice was moderate to high. In human breast cancer MDA MB-231, tumour uptake was similar at 1 and 2 hours post injection with 3.2±0.6 and 3.4±0.7 %ID/g, respectively (3–4 mice/group). In the mouse mammary tumour 4T1 uptake at 1 and 2 h (3 mice each), was 3.5±0.4 and 2.7±0.3%ID/g, respectively (results not shown). In the two human ovarian cancer xenografts IGROV-1 and SKOV-3 uptake at 1 h was 5.8±1.0 and 1.6±0.4 %ID/g (3 to 5 mice/group), respectively.

MicroPET dynamic imaging of A431 tumour bearing mice showed rapid tumour uptake within 10 min after injection followed by a slow release (result not shown). Imaging 1 h after injection of a mouse bearing on the right side an A431 tumour and on the left a 4T1 tumour showed significant tracer accumulation in A431 while activity in 4T1 remained in the range of abdominal activity (▶Fig. 5b).

Chase experiments in vivo

With the intention to reveal a supposed low affinity binding of ^{68}Ga-NODAGA-RGDyK in normal tissues like liver, kidneys and intestines, we performed chase experiments. We injected first ^{68}Ga-NODAGA-RGDyK i. v., followed 15 min later by the i. v. injection of moderate amounts (0.1 to 2.0 mg/kg) unlabeled NODAGA-RGDyK. We observed in a first experiment at 0.1 and

a

target organ	organ doses (mGy/MBq)	
	male	female
adrenals	0.0026	0.0033
brain	0.0011	0.0013
breasts	0.0012	0.0015
gallbladder wall	0.0037	0.0045
LLI wall	0.0065	0.0076
small intestine	0.0174	0.0203
stomach wall	0.0075	0.0087
ULI wall	0.0132	0.0151
heart wall	0.0038	0.0049
kidneys	0.0267	0.0291
liver	0.0238	0.0319
lungs	0.0040	0.0051
muscle	0.0024	0.0035
ovaries		0.0047
pancreas	0.0027	0.0034
red marrow	0.0032	0.0037
osteogenic cells	0.0029	0.0041
skin	0.0013	0.0015
spleen	0.0133	0.0162
testes	0.0022	
thymus	0.0014	0.0018
thyroid	0.0012	0.0014
urinary bladder wall	0.1390	0.1880
uterus		0.0065
total body	0.0033	0.0042
ED (mSv/MBq)	**0.012**	**0.016**

L(U)LI: lower (upper) large intestine

b

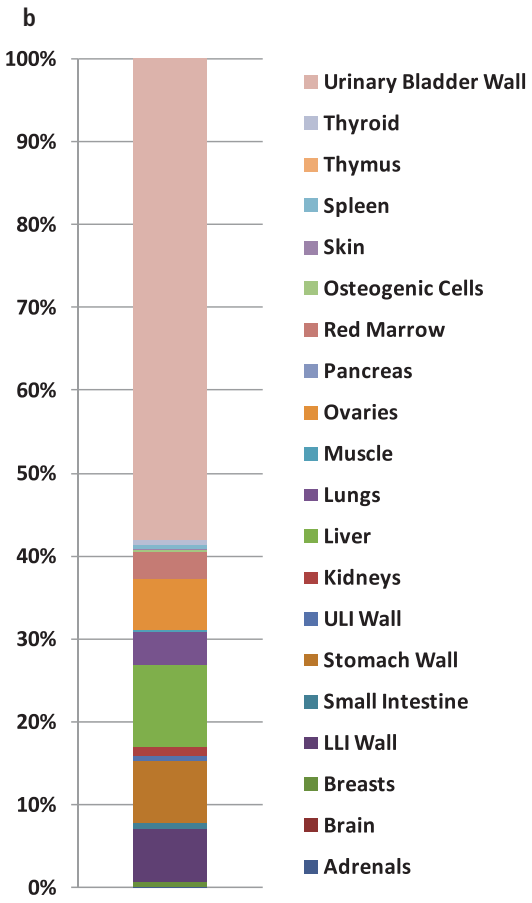


Fig. 3
Dosimetry extrapolation of ^{68}Ga-NODAGA-RGDyK for human beings as calculated from biodistribution studies in mice (Fig. 2) and assuming a bladder voiding interval of 0.5 h is shown.
a) Organ radiation doses and ED for males (70 kg) and females (58 kg), respectively, were obtained by OLINDA dosimetry.
b) Relative organ contributions to the ED in %.

1 mg/kg chase peptide a reduced ^{68}Ga-NODAGA-RGDyK uptake in tumour and adrenals but no uptake inhibition in other normal tissues (▶Fig. 6a).

In a second experiment, the chase with unlabeled peptide at 2 mg/kg given once or twice, showed decreased activity uptake in all tissues compared with controls, however uptake in tumour and adrenals was still reduced to a stronger degree (▶Fig. 6b).

In a third chase experiment biodistribution was measured two hours after injection of ^{68}Ga-NODAGA-RGDyK, chase being performed with 2×0.4 mg/kg unlabeled peptide injected at 15 and 60 minutes. It showed only a modest uptake decrease in tumour and adrenals as compared with unchased controls again without any modification in the other normal mouse tissues (▶Fig. 6c).

Discussion

NODAGA-RGDyK has been synthesized in GMP quality and is currently under review for a clinical study. Towards this aim, we selected from literature (6, 12) the components NODAGA for stable chelation of ^{68}Ga and pentacyclic RGDyK for $\alpha_v\beta_3$ integrin targeting. In RGDyK a single amino acid DTyr is replacing DPhe as compared with the original compound present in NODAGA-RGD (c(RGDfK)) of which the synthesis has been described (6, 12). In the meantime the stability of Ga and Cu chelation with NODAGA has also been confirmed by another group (7, 8).

Our in vitro and in vivo results entirely confirmed the published reliable radiolabeling of NODAGA-RGDyK with ^{68}Ga and the tumour localization capacity of this radioconjugate. As was also recently reported, ^{68}Ga-NODAGA-RGD behaved similarly as ^{18}F-galacto-RGD as a reference reagent (20). In agreement with these authors, we consider ^{68}Ga-NODAGA-RGDyK a promising agent for PET imaging of neovascular and tumour $\alpha_v\beta_3$ integrin expression. The dosimetry projection and absence of toxicity of this peptide at 1000-fold excess amount further confirmed its potential clinical usefulness.

Dosimetry results showed that bladder emptying intervals of 0.5 h optimally reduced bladder wall irradiation. However, bladder wall radiation dose still contributes to close to 60% of the ED as revealed by OLINDA dosimetry. It has been calculated by MonteCarlo simulations that further bladder wall dose reduction could be obtained by injection of patients without initial bladder voiding (9). Up to 80% bladder wall radiation dose reduction was observed when assuming an initial bladder filling with 0.3 l as compared with the injection of patients with an empty bladder (9).

We selected different tumours available at our institute for uptake studies with ^{68}Ga-NODAGA-RGDyK. While all tumour lines except one (A431), have been described to express integrin $\alpha_v\beta_3$, A431 only expresses $\alpha_v\beta_5$ (19). However, $\alpha_v\beta_5$ is probably also bound by RGDyK (21). Furthermore, when grafted into mice, tumour stroma and notably neovessels can express $\alpha_v\beta_3$ quite abundantly. When

tissue	mouse					human, residence time	
	g	%ID/g (t = 0)	% ID/organ (t = 0)	T 1/2 (h)	residence time	male	female
liver	1.19	4.84	5.76	1.05	0.0853	0.0853	0.0853
kidneys	0.41	6.65	2.73	0.43	0.0164	0.0164	0.0164
lung	0.24	4.69	1.13	0.43	0.0068	0.0068	0.0068
spleen	0.1	3.84	0.38	0.89	0.0048	0.0048	0.0048
heart	0.14	2.79	0.39	0.33	0.0018	0.0018	0.0018
muscle	9.73*	1.84	17.90	0.30	0.0767	0.0767	0.0767
bone	2.78**	2.23	6.20				
skin	5.56***	3.42	19.01				
stomach	0.41	2.46	1.01	0.43	0.0061	0.0061	0.0061
small intestine	1.34	2.57	3.44	0.58	0.0281	0.0281	0.0281
large intestine (LI)	0.94	2.42	2.27	0.39	0.0123		
upper LI						0.0103	0.0103
lower LI						0.0020	0.0020
blood	2.78†	3.87	10.76	0.36	0.0548		
red marrow						0.0083	0.0101
carcass	21.67	2.81	60.89				
bladder					0.12††	0.1200	0.1200
total body	27.8		80.79	0.41	**0.4701**		
rest body						0.1035	0.1017
(total residence time)						**0.4701**	**0.4701**

*35%, **10%, ***20%, †10% of mouse body weight; ††Bladder residence time was calculated with Mirdose bladder model: elimination fraction 0.75, biological half-life 0.67h, bladder voiding interval 0.5h.
Red marrow activity concentration is set identical to blood, respecting respective volumes in male and female human beings (19). Rest body residence time is equal to the total body residence time minus all organ residence times introduced into Olinda.

Tab. 1 Dosimetry extrapolation to humans from mouse biodistribution given in Figure 2, extrapolated half-lives, calculated residence times and repartition to human organs and tissues.

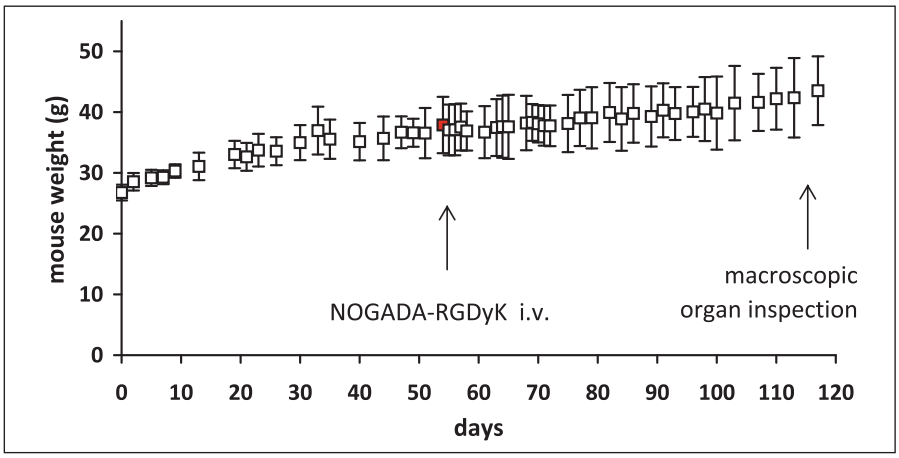


Fig. 4 Tolerance study of NODAGA-RGDyK in excess amount in mice is shown. Evolution of animal weights (n = 5) from arrival to the laboratory until injection of excess amounts of NODAGA-RGDyK (1000-fold excess per kg body weight) and the subsequent two months of observation are presented. **Note:** Absence of toxicity was shown by repeated inspection of the mice over the first five hours after injection, a normal weight curve evolution over two months (Fig. 4) and a normal organ appearance at necropsy.

looking at our results, low tumour uptake was observed in SKOV-3 tumours that expresses $\alpha_v\beta_3$, according literature. Stronger tumour uptake of ^{68}Ga-NODAGA-RGDyK was, however, observed in the other tumours, namely A431 and the breast and ovarian cancers MDA-MB-231 and IGROV-1. These results therefore suggest that tumour uptake of RGDyK may depend or co-depend from other factors than $\alpha_v\beta_3$ integrin expression by the tumour lines themselves.

As also observed with other $\alpha_v\beta_3$ targeting reagents, significant background activity was observed in liver, kidneys, the gastrointestinal tract and other normal tissues. Uptake in these normal organs was reduced significantly when using co-injection of excess (5 mg/kg) amount of

unlabeled peptide with ^{68}Ga-NODAGA-RGDyK. Such similar observations made by other groups led to the hypothesis of a low affinity binding to integrins other than αvβ3 in normal mouse tissues. We performed a small series of chase experiments whereby moderate amounts of NODAGA-RGDyK were injected i. v. 15 min post ^{68}Ga-NODAGA-RGDyK injection. Overall, the results showed that the low amounts of RGDyK were not able to displace ^{68}Ga-NODAGA-RGDyK localization from most normal tissues except adrenals and tumour A431. These results are suggestive of a different binding mode of NODAGA-RGDyK to most normal tissues as compared with tumour and adrenals.

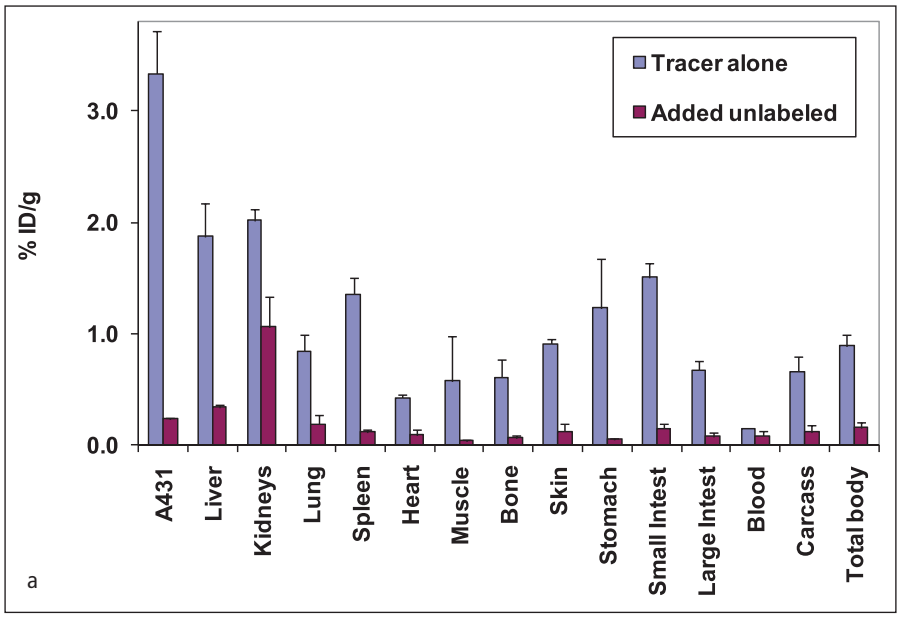
Interestingly, another group observed a particular stable localization of ^{64}Cu-NODAGA-c(RGDfK) in tumour and adrenals (7). They mentioned that $\alpha_v\beta_3$ was also expressed by normal adrenal tissue (17) similar as by tumour which could explain the particular stability of the RGD peptide uptake in these two tissues.

Conclusion

The predicted moderate ED and EDE of 0.014 and 0.018 mSv/MBq, respectively, and the observed tumour uptake and retention qualify ^{68}Ga-NODAGA-RGDyK as a promising PET tracer targeting $\alpha_v\beta_3$ integrin.

> We observed an easy to perform and reliable radiolabeling of NODAGA-RGDyK. NODAGA-RGDyK was also well tolerated in excess amount.

The chase experiments showed that tumour and adrenal ^{68}Ga-NODAGA-RGDyK uptake could be selectively reduced, suggestive of a reversible, specific binding in these tissues, while no chase was observed at low NODAGA-RGDyK concentrations in normal tissues other than adrenals, deserving further investigations.



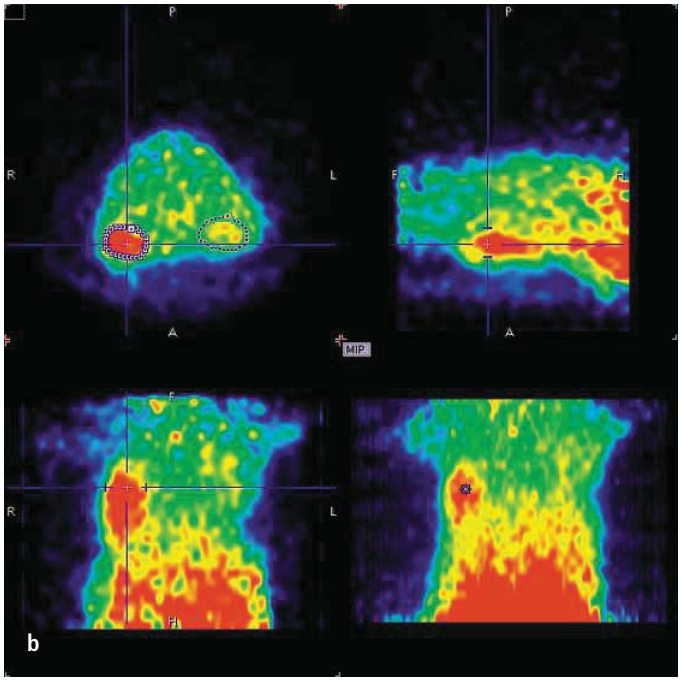


Fig. 5 Representative biodistribution study (**a**) and microPET imaging (**b**) at one hour after i. v. injection of ^{68}Ga-NODAGA-RGDyK in SCID mice bearing an A431 human and a 4T1 mouse tumour xenografts

a) Result of a co-injection of ^{68}Ga-NODAGA-RGDyK with unlabeled NODAGA-RGDyK (5 mg/kg) shows uptake inhibition in tumour and in most normal organs.

b) PET imaging shows the three sections in the orthogonal planes relative to the A431 tumour (crossection mark): transverse section (upper left), sagittal section (upper right), coronal section (lower left) and the dorsal plain view of the animal (lower right) with the anterior and hind legs at the border of the field of view of microPET. r, right; l, left; a, anterior; p, posterior; f, front and h, hind of the animal. The marked crossection and major circled hyperactivity shows on the right anterior side the subcutaneous A431 tumour with strong uptake of ^{68}Ga-NODAGA-RGDyK. The 4T1 mouse mammary tumour on the left anterior side appears iso-active with the abdomen indicating moderate uptake of ^{68}Ga-NODAGA-RGDyK.

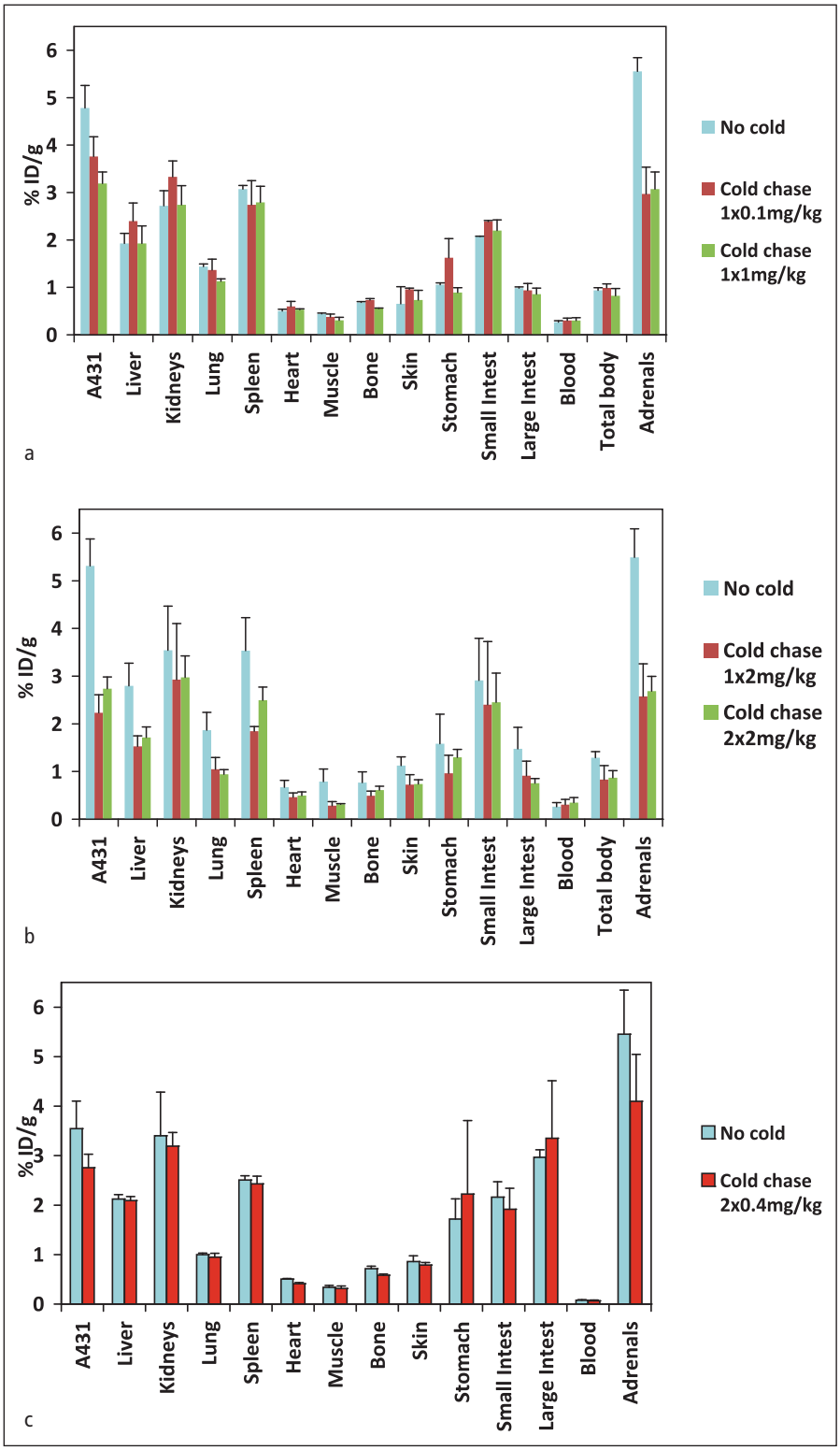


Fig. 6 Chase experiments 1 to 3 (in %ID/g): ^{68}Ga-NODAGA-RGDyK was injected i. v. and **a)** chased or not 15 min later with the i. v. injection of 0.1 to 1 mg/kg NODAGA-RGDyK (biodistribution measured 1 h after injection)
b) chased once or twice (at 15 and 30 min) with 2 mg/kg NODAGA-RGDyK (biodistribution measured 1 h after injection).
c) Animals were chased with 2×0.4 mg/kg NODAGA-RGDyK i. v. at 15 and 60 min post radiotracer injection and biodistribution measured at two hours.

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Conflict of interest

The authors declare, that there is no conflict of interest.

References

1. Beer AJ, Haubner R, Goebel M et al. Biodistribution and pharmacokinetics of the alphavbeta3-selective tracer 18F-galacto-RGD in cancer patients. J Nucl Med 2005; 46: 1333–1341.
2. Beer AJ, Haubner R, Wolf I et al. PET-based human dosimetry of ^{18}F-galacto-RGD, a new radiotracer for imaging alpha v beta3 expression. J Nucl Med 2006; 47: 763–769.
3. Benard J, Da Silva J, De Blois MC et al. Characterization of a human ovarian adenocarcinoma line, IGROV1, in tissue culture and in nude mice. Cancer Res 1985; 45: 4970–4979.
4. Chen X, Park R, Tohme M et al. MicroPET and autoradiographic imaging of breast cancer alpha v-integrin expression using ^{18}F- and ^{64}Cu-labeled RGD peptide. Bioconjugate Chem 2004; 15: 41–49.
5. Cruet-Hennequart S, Maubant S, Luis J et al. alpha(v) integrins regulate cell proliferation through integrin-linked kinase (ILK) in ovarian cancer cells. Oncogene 2003; 22: 1688–1702.
6. Decristoforo C, Hernandez Gonzalez I, Carlsen J et al. ^{68}Ga- and ^{111}In-labelled DOTA-RGD peptides for imaging of alphavbeta3 integrin expression. Eur J Nucl Med Mol Imaging 2008; 35: 1507–1515.
7. Dumont RA, Deininger F, Haubner R et al. Novel ^{64}Cu- and ^{68}Ga-labeled RGD conjugates show improved PET imaging of {alpha}{nu}{beta}3 integrin expression and facile radiosynthesis. J Nucl Med 2011; 52: 1276–1284.
8. Fani M, Del Pozzo L, Abiraj K et al. PET of somatostatin receptor-positive tumors using ^{64}Cu- and ^{68}Ga-somatostatin antagonists: The chelate makes the difference. J Nucl Med 2011; 52: 1110–1118.
9. Freudenberg R, Oehme L, Menkel S et al. Strahlenbelastung der Blasenwand nach nuklearmedizinischer Radiopharmakaapplikation – Anwendung des MIRD Pamphlet 14. Nucl Med 2011; 50: A112.
10. Heckmann D, Kessler H. Design and chemical synthesis of integrin ligands. Methods Enzymol 2007; 426: 463–503.
11. Jeong JM, Hong MK, Chang YS et al. Preparation of a promising angiogenesis PET imaging agent: ^{68}Ga-labeled c(RGDyK)-isothiocyanatobenzyl-1,4,7-triazacyclononane-1,4,7-triacetic acid and feasibility studies in mice. J Nucl Med 2008; 49: 830–836.
12. Knetsch PA, Petrik M, Griessinger CM et al. [^{68}Ga]NODAGA-RGD for imaging alpha(v)beta(3) integrin expression. Eur J Nucl Med Mol Imaging 2011; 38: 1303–1312.

13. Li ZB, Cai W, Cao Q et al. ^{64}Cu-labeled tetrameric and octameric RGD peptides for small-animal PET of tumor alpha(v)beta(3) integrin expression. J Nucl Med 2007; 48: 1162–1171.
14. Li ZB, Chen K, Chen X. ^{68}Ga-labeled multimeric RGD peptides for microPET imaging of integrin alpha(v)beta(3) expression. Eur J Nucl Med Mol Imaging 2008; 35: 1100–1108.
15. Liu Z, Liu S, Wang F et al. Noninvasive imaging of tumor integrin expression using ^{18}F-labeled RGD dimer peptide with PEG (4) linkers. Eur J Nucl Med Mol Imaging 2009; 36: 1296–1307.
16. Luo BH, Carman CV, Springer TA. Structural basis of integrin regulation and signaling. Ann Review Immunol 2007; 25: 619–647.
17. Otis M, Campbell S, Payet MD et al. In adrenal glomerulosa cells, angiotensin II inhibits proliferation by interfering with fibronectin-integrin signaling. Endocrinology 2008; 149: 3435–3445.
18. Pettinato C, Sarnelli A, Di Donna M et al. ^{68}Ga-DOTANOC: biodistribution and dosimetry in patients affected by neuroendocrine tumors. Eur J Nucl Med Mol Imaging 2008; 35: 72–79.
19. Pidgeon GP, Tang K, Cai YL et al. Overexpression of platelet-type 12-lipoxygenase promotes tumor cell survival by enhancing alpha(v)beta(3) and alpha(v)beta(5) integrin expression. Cancer Res 2003; 63: 4258–4267.
20. Pohle K, Bussemer J, Schwaiger M et al. Imaging of alpha-v-beta-3 expression: A comparative study using [^{18}F]galacto-RGD and [^{68}Ga]NODAGA-RGD. J Nucl Med 2011; 52: 120P.
21. Reardon DA, Neyns B, Weller M et al. Cilengitide: an RGD pentapeptide alphavbeta3 and alphavbeta5 integrin inhibitor in development for glioblastoma and other malignancies. Future Oncol 2011; 7: 339–354.
22. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. J Nucl Med 2005; 46: 1023–1027.
23. Stabin MG. MIRDOSE: personal computer software for internal dose assessment in nuclear medicine. J Nucl Med 1996; 37: 538–546.
24. Stewart PL, Nemerow GR. Cell integrins: commonly used receptors for diverse viral pathogens. Trends Microbiol 2007; 15: 500–507.
25. Wang W, Luo BH. Structural basis of integrin transmembrane activation. J Cell Biochem 2010; 109: 447–452.