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Synthesis and biological evaluation of ^{68}Ga labeled bis-DOTA-3,3′-(benzylidene)-bis-(1*H*-indole-2-carbohydrazide) as a PET tracer for in vivo visualization of necrosis

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ABSTRACT

The aim of the present study was to develop a ^{68}Ga labeled bis-DOTA derivative of benzylidene-bis-indole and compare the in vivo stability and biodistribution with that of the previously reported bis-DTPA derivate for in vivo imaging of necrosis using PET. Uptake of the tracer was evaluated in a mouse model of Fas-mediated hepatic apoptosis in correlation with histochemical stainings. The novel ^{68}Ga labeled tracer showed an improved in vivo stability and could therefore be used for selective non-invasive imaging of necrotic cell death using PET.

Cell death is typically dichotomously discussed as apoptosis and necrosis.[1–3] Apoptosis can be described as an active, genetically programmed process of autonomous cellular dismantling that plays an essential role in organ development, tissue homeostasis and removal of defective cells. In contrast, necrosis has often been referred to as an accidental form of cell death. This was consistent with a rapid swelling of the cell, loss of cell membrane integrity and release of intracellular contents that are observed as a consequence of overwhelming physiochemical stress to the cell. Recent evidence, however, has changed the perception of necrotic cell death. Depending on the context, necrotic cell death might be fully unregulated or 'programmed' as necrosis is now no longer considered to be a passive consequence but a process which the cell can control.[4–6] Both apoptosis and necrosis can occur simultaneously in pathological conditions, although their specific contribution to a pathological process is often still poorly understood, with excessive cell death resulting in a progressive loss of tissue functionality as it occurs in acute myocardial infarction, stroke, neurodegenerative disorders and atherosclerosis.[7,8] Given the significant role of cell death in a variety of pathologies and the potential therapeutic applications of cell death modulation, noninvasive monitoring of the rate and extent of cell death could provide relevant information on disease progression and therapy response; thereby assist in the diagnosis and therapy management.[9] Several positron emission tomography (PET) tracers have recently been proposed for in vivo imaging of apoptosis including ^{11}C or ^{18}F labeled isatin sulfonamide analogs that exhibit nanomolar affinity for activated caspase 3 or 7, hall mark intracellular biomarkers for apoptosis, and ^{18}F labeled ML-10 (2-(5-fluoro-pentyl)-2-methyl-malonic acid, MW = 206 Da) which was reported to show selective uptake and accumulation in apoptotic cells.[10–12] Radiotracers for imaging of necrosis often exploit loss of membrane integrity which allows biomolecules, that normally are concentrated intracellularly, to interact with the extracellular environment. However, the most commonly used positron emitters, that is ^{11}C and ^{18}F, are cyclotron produced and due to their short half-life require an expensive on-site cyclotron for tracer production. A generator-based PET radioisotope such as gallium-68 ($t_{1/2}$ = 68 min, electron capture = 11%, β^+ = 89%, E_{max} of positron = 1.9 MeV), that can simply be eluted from a ^{68}Ge/^{68}Ga generator system ($t_{1/2}$ ^{68}Ge = 270.8 days), provides an alternative for preparation and availability of PET-tracers.[13]

Previously, our group successfully synthesized a benzylidene-bis-indole derivative, ECIV-7, a bis-gadolinium labeled *N*,*N*′-bis(diethylenetriamine pentaacetic acid)-3,3′-(benzylidene)-bis-(1*H*-indole-2-carbohydrazide) (Gd_2-bis-DTPA-BI) which was found

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to concentrate in necrotic tissue.[14] The presence of the acyclic chelator DTPA allowed labeling of the compound with ^{68}Ga for use as a tracer agent in PET. Although the resulting complex displayed a selective uptake in different animal models of necrosis, the tracer suffered from a limited in vivo stability, probably because DTPA is not an ideal chelator for ^{68}Ga,[15] resulting in an unfavorable biodistribution and high blood pool activity. Therefore the aim of the present study was the development of a ^{68}Ga labeled benzylidene-bis-indole derivative with improved in vivo stability and biodistribution, and evaluate the resulting complex as a potential PET tracer for imaging of necrotic cell death.

Starting from the compound Gd_2-ECIV-7 described by Ni et al.,[14] a bis-DOTA derivate of benzylidene-bis-indole (compound **4**; bis-DOTA-BI) was synthesized in high purity via a three-step synthesis (Fig. 1) with an overall yield of 45.5%.[16] Labeling with generator produced ^{68}Ga in biocompatible sodium acetate buffer was straightforward and fast[17] (radiochemical purity after HPLC-purification >98%; decay-corrected radiochemical yield: 43.1 ± 6.5%; specific activity 12 GBq/μmol).

Comparison of the biodistribution of ^{68}Ga-bis-DOTA-BI with that of the ^{68}Ga labeled bis-DTPA analog 30 min and 4 h post injection (pi) in normal NMRI mice shows that blood washout of ^{68}Ga-bis-DOTA-BI was 6.8 times faster than the washout of the previously reported DTPA analog (Table 1).[18] This difference in blood washout could be explained by a possible in vivo transchelation of ^{68}Ga from the less stable ^{68}Ga-DTPA complex to transferrin, leading to a longer retention of ^{68}Ga activity in the blood. The higher in vivo stability of the novel tracer as compared to the DTPA analog was confirmed by reversed phase HPLC analysis of plasma from normal NMRI mice after intravenous injection of the tracer. Table 2 shows the percentage of intact ^{68}Ga-bis-DOTA-BI in plasma of normal NMRI mice as a function of time, indicating that the tracer has a reasonable in vivo stability in plasma when compared to the DTPA analog with 43.4% and 12.5% of intact tracer at 240 min pi for ^{68}Ga-bis-DOTA-BI and the DTPA analog, respectively.

Table 2
Percentage of intact ^{68}Ga-bis-DOTA-BI and intact ^{68}Ga-bis-DTPA in plasma of normal NMRI mice at 10, 30, 90 and 240 min pi (n = 2 per time point)

Time point (min)	% Intact ^{68}Ga-DOTA-BI	% Intact ^{68}Ga-DTPA-BI
10	94.6 ± 0.6	87.8 ± 0.4
30	87.7 ± 0.4	64.1 ± 0.4
90	76.7 ± 0.3	n.d.
240	43.4 ± 0.3	12.5 ± 0.5

Data are expressed as mean ± SD; n.d.: not determined.

Ex vivo autoradiography of the liver of rats with a reperfused partial liver infarction revealed that the tracer displays selective uptake in areas of necrosis (as confirmed by histochemical staining) with necrotic tissue to viable tissue activity ratios of 10 and 12 at 30 and 90 min pi, respectively (Fig. 2).[19]

Dynamic microPET images were acquired to compare the kinetics of the tracer in necrotic and viable liver tissue as well as to quantify the uptake of the tracer.[20] The microPET images (Fig. 3) confirmed uptake of the new tracer in the necrotic liver lobes. As the tracer showed a faster blood washout and less retention in other organs than in the target tissue when compared to the DTPA analog, the imaging contrast between target and non-target tissue was higher at earlier time points which is important in case of acquisition of images using short-lived radioisotopes such as ^{68}Ga.

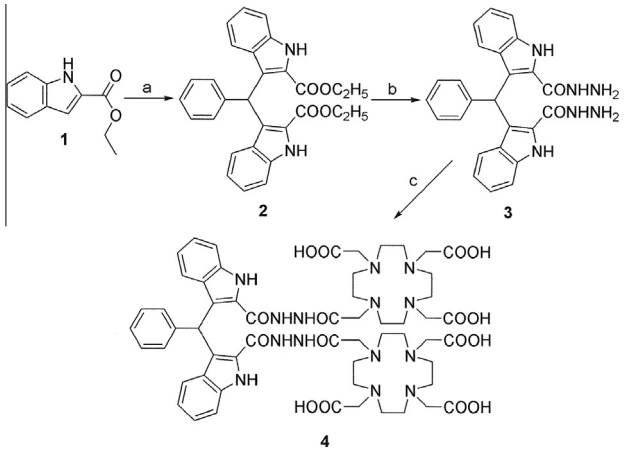

Figure 1. Synthesis scheme of bis-DOTA-BI (4). Reagents and conditions: (a) benzaldehyde, 37% HCl, EtOH, N_2 atm, reflux 2 h; (b) $NH_2NH_2{\cdot}H_2O$, Pyr/MeOH, reflux (48 h); (c) DOTA-NHS, DIEA, DMF, rt 24 h.

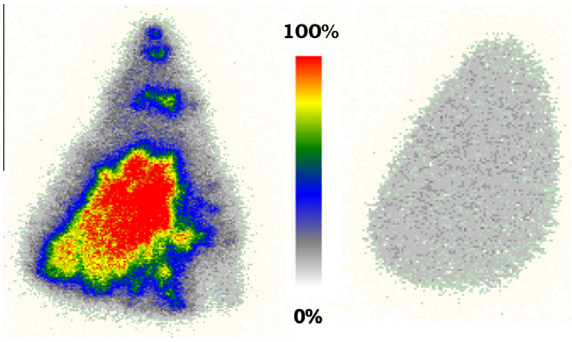

Figure 2. Ex vivo autoradiographic images of 50-μm sections of necrotic (left) and viable (right) liver lobes of a Wistar rat with reperfused partial liver infarction 90 min pi of ^{68}Ga-bis-DOTA-BI.

Table 1
Comparison of the biodistribution of ^{68}Ga-bis-DOTA-BI and ^{68}Ga-bis-DTPA-BI in normal NMRI mice at 30 and 240 min pi ($n \geqslant 4$/time point)

Organ	^{68}Ga-bis-DOTA-BI (% of ID[a])		^{68}Ga-bis-DTPA-BI (% of ID[a])	
	30 min	240 min	30 min	240 min
Urine	41.1 ± 5.1	87.7 ± 1.1	32.1 ± 7.0	57.0 ± 4.9
Kidneys	3.9 ± 0.4	1.6 ± 0.3	3.8 ± 1.5	2.7 ± 0.4
Liver	3.1 ± 0.3	1.2 ± 0.2	3.4 ± 0.4	3.3 ± 0.5
Spleen and pancreas	0.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
Lungs	1.3 ± 0.4	0.2 ± 0.0	0.7 ± 0.2	0.7 ± 0.2
Heart	0.3 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
Intestines and feces	2.8 ± 0.3	4.9 ± 0.9	3.2 ± 0.5	7.2 ± 1.7
Stomach	0.4 ± 0.1	0.3 ± 0.2	0.5 ± 0.1	0.7 ± 0.3

Data are expressed as mean ± SD.
[a] Percentage of injected dose calculated as cpm in tissue/total cpm recovered in all parts of animals.