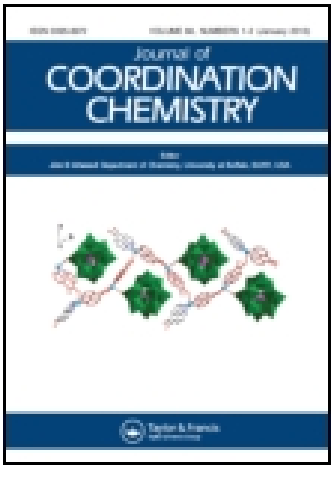

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Synthesis and properties of neutral gadolinium and technetium-99m-labeled complexes

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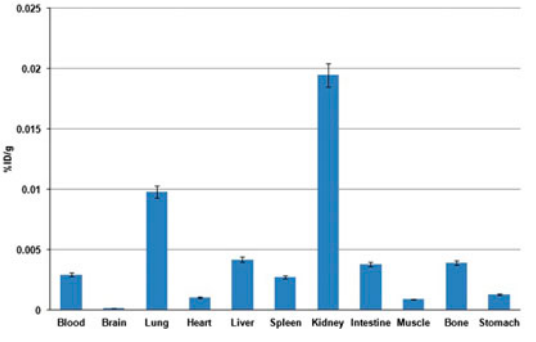

Diethylenetriaminepentaacetic di(L-dopa ethyl ester) ligand (DTPA-2LDEE) was synthesized by reaction between diethylenetriaminepentaacetic dianhydride (DTPAA) and L-dopa ethyl ester (LDEE). This ligand reacted with gadolinium chloride and sodium pertechnetate to make the corresponding neutral gadolinium complex Gd-DTPA-2LDEE and technetium-99m-labeled complex ^{99m}Tc-DTPA-2LDEE, respectively. The ligand and complexes were characterized and their properties *in vitro* and *in vivo* were also evaluated. Gd-DTPA-2LDEE possessed higher relaxation effectiveness and lower cytotoxicity to human hepatoma HepG-2 cells than Gd-DTPA. ^{99m}Tc-DTPA-2LDEE had enhanced single-photon emission computed tomography (SPECT) signal enhancements of the kidneys in rats and provided longer duration time than that of ^{99m}Tc-DTPA. Therefore, ^{99m}Tc-DTPA-2LDEE can be selectively excreted by the kidneys and used as a potential radioactive probe in SPECT.

Keywords: Single-photon emission computed tomography (SPECT); Magnetic resonance imaging (MRI); Gadolinium complex; Technetium-99m-labeled complex; Diethylenetriaminepentaacetic acid; L-dopa ethyl ester

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1. Introduction

Polyaminopolycarboxylic acids have been widely used as ligands to form metal complexes which are water soluble and kinetically inert under various conditions and have broad application as contrast agents in magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) imaging, etc. [1, 2].

MRI is a noninvasive clinical imaging modality, widely used in diagnosis of human diseases. Water-soluble MRI contrast agents are a unique class of pharmaceuticals that consist of paramagnetic metal ions and ligand that can enhance the image contrast of MRI between normal and diseased tissue [3–8]. Gadolinium(III) polyaminopolycarboxylic acid chelates, such as gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) [9], diethylenetriaminepentaacetic acid-bismethylamide (Gd-DTPA-BMA) [10], and gadolinium 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acids (Gd-DOTA) [11], are in current clinical use as MRI contrast agents.

SPECT is a nuclear imaging technique which is based on detection of radiation emitted by an intravenously injected radionuclide-labeled probe that emits single photons [12]. Currently, it provides 3-D, noninvasive, quantitative images of the distribution of radiotracers used to mark physiological, metabolic, or pathological processes in humans or animals. Compared with other imaging methods, SPECT suffers from a lower spatial resolution and requires longer time for image acquisition. A number of radionuclides decay with γ emissions that are suitable for improved imaging contrast, such as gallium-67 (^{67}Ga), iodine-123 (^{123}I), indium-111 (^{111}In), technetium-99m (^{99m}Tc), and thallium (^{201}Tl). Technetium-99m has been a prevalently used radionuclide for diagnostics based on its favorable nuclear properties, including a single 140 keV photon emission (ideal for most γ cameras), absence of particle emission, a half-life of 6.03 h, etc. Commonly used complexes in clinic, such as technetium-99m diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) and technetium-99m 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (^{99m}Tc-DOTA), are prepared by coordination of technetium-99m with the corresponding DTPA and DOTA, respectively [12–16].

However, the clinically used metal-diethylenetriaminepentaacetic acid complexes, such as Gd-DTPA and ^{99m}Tc-DTPA, are small ionic molecules that can diffuse freely through the extracellular space and be excreted rapidly by the kidneys; their biodistributions are nonspecific and duration times are about 30 min in the body. Injection of large quantities of the ionic complex will raise ion concentration *in vivo* and cause localized disturbances in osmolality, which, in turn, leads to cellular and circulatory damage [17–19]. An ideal molecular imaging probe should be designed as tissue- or organ-targeting neutral materials with high relaxivity, low toxicity and side effects, suitable duration and excretion time, and high imaging contrast enhancement with low dose *in vivo* and low overall cost [20–24].

Dopamine, as a catecholamine neurotransmitter, plays important roles in the behavior and cognition, voluntary movement, motivation, punishment and reward, and inhibition of prolactin production in the brain. L-Dopa (3,4-dihydroxyphenyl-L-alanine) is a precursor of dopamine (DA) which is deficient in the brains of patients suffering from the progressive disorder of the central nervous system (CNS) known as Parkinson's disease (PD). L-Dopa can be considered a prodrug of DA since L-Dopa enters into the CNS through active transport and it is enzymatically decarboxylated in the brain giving rise to DA. However, L-Dopa has low water solubility, sensitivity to chemical and enzymatic oxidations, and suffers from extensive peripheral decarboxylation [25–28].

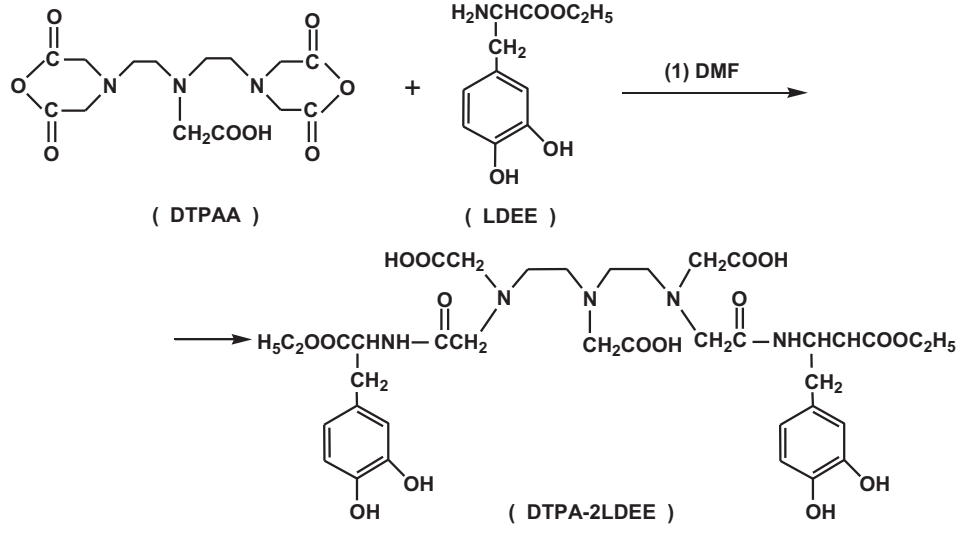
In this work, L-dopa ethyl ester (LDEE) was incorporated to DTPA to synthesize diethylenetriaminepentaacetic di(L-dopa ethyl ester) ligand (DTPA-2LDEE). This ligand was further reacted with gadolinium chloride and sodium pertechnetate to make the corresponding water-soluble neutral gadolinium complex Gd-DTPA-2LDEE and technetium-99m-labeled complex ^{99m}Tc-DTPA-2LDEE, respectively (scheme 1). The ligand and metal complexes were characterized and their properties *in vitro* and *in vivo* were also evaluated.

2. Experimental

2.1. *Materials and instrumentation*

These compounds were characterized using a Nicolet IS10 Fourier transform infrared (FT-IR) spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI, United States of America), a UV–Vis spectrophotometer (UV-2800 series, Unico, Shanghai, China), a Varian Mercury-VX300 NMR spectrometer (Varian, Inc. Corporate, Palo Alto, CA, United States of America), and a Voyager DE STR MALDI-TOF-MS Laser Time-of-Flight Mass Spectrometer (Applied Biosystems by Life Technologies Co., United States of America). The concentration of the paramagnetic species [Gd^{3+}] was measured by an Intrepid XSP Radial inductively coupled plasma emission spectrometer (ICP-AES, IRIS Intrepid II, Thermo Fisher Scientific Inc., Madison, WI, United States of America). The solvent longitudinal relaxation time (T_1) for gadolinium complex in distilled water was determined by a Varian Mercury-VX300 NMR spectrometer. The SPECT imaging was carried out on a SYMBIA T6 SPECT/CT scanner (Siemens, Germany).

Eight normal SD white rats (weight: 75 g) were provided by the School of Pharmacy (Tongji Medical College, Huazhong University of Science and Technology, China) and were cultured according to the method described in the literature [29]. The human hepatoma



Scheme 1. Synthetic route to diethylenetriaminepentaacetic di(L-dopa ethyl ester) (DTPA-2LDEE).

HepG-2 cells were provided by the China Center for Type Culture Collection of Wuhan University, China. The ethical approval was obtained for *in vivo* experiments in rats from the Department of Science and Technology of Hubei Province, China, and the Animal Center of Tongji Medical College, Huazhong University of Science and Technology, China.

All chemicals and solvents were of analytical grade. DTPA dianhydride (DTPAA), gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) [30–32], and LDEE hydrochloride [33] were synthesized according to the methods established.

2.2. *Synthesis of ligand*

L-dopa ethyl ester hydrochloride (LDEE.HCl, 0.523 g, 0.002 M) was dissolved in DMF (5 mL) and cooled to 0 °C in an ice–salt bath. The solution was purged by nitrogen and adjusted pH to 9 by triethylamine. A solution of diethylenetriaminepentaacetic dianhydride (DTPAA, 0.357 g, 0.001 M) in DMF (10 mL) was added slowly to the solution of LDEE in DMF with rapid stirring. The reaction continued stirring for 8 h at 0 °C and a further 48 h at room temperature. The resultant mixture was filtered and precipitated with acetonitrile (100 mL). The precipitate was reprecipitated from DMF using acetonitrile, filtered and dried under vacuum to yield a white solid. The solid was purified on a silica column to afford diethylenetriaminepentaacetic di(L-dopa ethyl ester) (DTPA-2LDEE, 0.638 g, 79%). ^{1}H NMR (D_2O, δppm): 6.58–6.41 (m, 6H, phenyl), 4.45 (2H, Ph–C**H$_2$**–NH), 3.91 (4H, 2 × C**H$_2$**–COOH), 3.68 (2H, C**H$_2$**–COOH), 3.36 (2H, 2 × C**H**–NH–CO), 2.88 (4H, 2 × N–C**H$_2$**), 2.61 (4H, 2 × N–C**H$_2$**). MS found $M + H^+$ 808.88 for $C_{36}H_{49}O_{16}N_5$, calcd 807; IR (KBr, ν_{max}, cm^{-1}): 3382 (OH), 2920 (C–H), 1645, 1527 (COO, CONH), 1410 (C–N), 1011 (C–O). UV (H_2O, λ_{max}, nm): 288.

2.3. *Synthesis of the gadolinium complex*

DTPA-2LDEE (1.024 g, 1.269 mM) was dissolved in methanol (12 mL) and then the solution of gadolinium chloride (0.471 g, 1.269 mM) in methanol (8 mL) was added. The reaction continued stirring for 4 h at room temperature. The resultant mixture was filtered and precipitated with anhydrous ether (100 mL). The precipitate was reprecipitated from methanol using ether, and then filtered and dried under vacuum to yield a white solid gadolinium diethylenetriaminepentaacetic di[L-dopa ethyl ester] (Gd-DTPA-2LDEE, 0.994 g, 81.5%). IR (KBr, ν_{max}, cm^{-1}): 3436 (OH), 2922 (C–H), 1632, 1600 (COO, CONH), 1405 (C–N), 1118, 1018 (C–O). UV (H_2O, λ_{max}, nm): 284. Gd-DTPA-2LDEE.H_2O: MS found M (.H_2O)+H^+ 979.62 for $GdC_{36}H_{48}O_{17}N_5$, calcd 979; Gd-DTPA-2LDEE: MS found $M + H^+$ 962.62 for $GdC_{36}H_{46}O_{16}N_5$, calcd 961.

2.4. *Synthesis of technetium-99m-labeled complex*

DTPA-2LDEE (3 mg, 1.269 mM) was dissolved in 0.9% sodium chloride solution (0.5 mL) and then acetic acid–sodium acetate buffer solutions (0.2 mL, 0.1 M/L, pH 4) was added. A solution of sodium pertechnetate ($Na^{99m}TcO_4$, 1 mL, 10 mCi) in 0.9% sodium chloride solution was added slowly to the solution of DTPA-2LDEE with rapid stirring at room temperature. Subsequently, a solution of stannous chloride (9 mg) and vitamin C (1.0 mg/mL) in hydrochloric acid (1 mL, 1 M/L) was added with rapid stirring at room temperature. The reaction solution continued stirring for 0.5 h at room temperature and was purified on a

Sephadex column chromatograph to yield a technetium-99m diethylenetriaminepentaacetic di[L-dopa ethyl ester] (^{99m}Tc-DTPA-2LDEE, 3.58 mCi/mL). IR (KBr, ν_{max}, cm^{-1}): 3440 (OH), 2930 (C–H), 1630, 1600 (COO, CONH), 1401 (C–N), 1110, 1014 (C–O). UV (H_2O, λ_{max}, nm): 286. ^{99m}Tc-DTPA-2LDEE.H_2O: MS found M(.H_2O)+H^+ 999.79 for $^{99m}TcC_{36}H_{48}O_{17}N_5$, calcd 999; ^{99m}Tc-DTPA-2LDEE: MS found $M + H^+$ 972.75 for $^{99m}TcC_{36}H_{46}O_{16}N_5$, calcd 971.

Technetium-99m diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA, 5 mCi/mL) was prepared by the reaction between diethylenetriamine-pentaacetic acid and sodium pertechnetate using the same method.

2.5. *Relaxivity*

In the absence of solute–solute interactions, solvent relaxation rates are linearly dependent on the concentration of paramagnetic species ([M]). Relaxivity, r_1, is defined as the slope of this dependence (1):

$$(1/T_1)_{obsd} - (1/T_1)_d = r_1[M] \quad (1)$$

where $(1/T_1)_{obsd}$ is an observed solvent relaxation rate in the presence of a paramagnetic species and $(1/T_1)_d$ is a solvent relaxation rate in the absence of a paramagnetic species. In this experiment, the concentrations of the paramagnetic species [Gd^{3+}] were measured by an Intrepid XSP Radial inductively coupled plasma emission spectrometer. The solvent longitudinal relaxation time (T_1) for gadolinium complex was carried out on 0.1–1.2 mM/L solution of gadolinium complex in distilled water. Thus, r_1 for gadolinium complex in distilled water could be calculated.

2.6. In vitro *cytotoxicity assay*

Human hepatoma HepG-2 cells (2×10^5/mL) were plated in 96-well plates in the growth medium (RPMI-1640 media: 10% fetal bovine serum (Gibco Co., USA), 100 units/mL penicillium, 100 μg/mL streptomycin) and the number of cells in each well was 2×10^4. The cells were incubated for 24 h in an incubator (37 °C, 5% CO_2) and the growth medium was then replaced with 100 μL of the growth medium containing gadolinium complex Gd-DTPA or Gd-DTPA-2LDEE. After 48 h incubation, 20 μL of MTT (thiazolyl blue (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 5.0 mg/mL) solution in the phosphate buffer saline solution (PBS) was added to each well. The cells were incubated for 3 h again and the growth medium was removed. About 100 μL of DMSO was then added and shaken for 30 min at room temperature. The optical density (OD_{472}) was measured at 472 nm with a DG-3022A ELISA-Reader and expressed as a percentage relative to control cells (no gadolinium complex).

2.7. *SPECT imaging*

SPECT imaging was carried out on a SYMBIA T6 SPECT/CT scanner (Siemens, Germany). The eight normal SD white rats (weight: 75 g) were anesthetized with chloral hydrate (0.35 mL/100 g, 10%) injected by intraperitoneal injection, positioned prone and fixed to a polystyrene cradle with adhesive tape to minimize respiratory motion. After

performing nonenhanced SPECT imaging, a solution of ^{99m}Tc-DTPA-2LDEE (0.35 mL, 1.2 mCi), ^{99m}Tc-DTPA (0.25 mL, 1.2 mCi) or $Na^{99m}TcO_4$ (1.2 mCi) was injected into the auricular vein. Static images of SD white rats were obtained at different time intervals post administration with a matrix size of 256 × 256, a zoom of 2.0, and an acquisition time of 10 min. Each rat was sacrificed at 15 h after receiving an injection of ^{99m}Tc-DTPA-2LDEE. Samples of blood, brain, lung, heart, liver, spleen, kidney, small intestine, muscle, and bone were taken. Tissues were blotted to remove excess blood, weighed, and analyzed by a SN-695 gamma counter RIA program ver. 5.33. Percentage of injected dose per organ (%ID/g) was calculated.

3. Results and discussion

3.1. *Synthesis and characterization*

Gd-DTPA-2LDEE and ^{99m}Tc-DTPA-2LDEE were synthesized by reaction of LDEE with diethylenetriaminepentaacetic dianhydrides and subsequent chelation with gadolium chloride and sodium pertechnetate, respectively. Gd-DTPA-2LDEE and ^{99m}Tc-DTPA-2LDEE were characterized by IR, MS, and UV. DTPA-2LDEE was also characterized by IR, MS, ^{1}H NMR, and UV.

^{1}H NMR spectra of DTPA-2LDEE showed the characteristic peaks of NCH_2COO of DTPA structure with 2.8–3.9 ppm and benzyl groups of LDEE with 6.58–6.41 ppm, indicating that DTPA was covalently bound to L-dopa ethyl ester. The MS spectra of DTPA-2LDEE displayed the correct peak of $M + H^+$ in accord with the molecular weight of DTPA-2LDEE (MW 807). IR spectra of free DTPA-2LDEE showed characteristic absorptions of carboxyl with 1645–1527 cm^{-1}; these peaks disappeared and strong absorptions at 1635–1600 cm^{-1} were present in IR spectra of Gd-DTPA-2LDEE and ^{99m}Tc-DTPA-2LDEE. The UV spectra of DTPA-2LDEE, Gd-DTPA-2LDEE, and ^{99m}Tc-DTPA-2LDEE demonstrated the characteristic peaks of L-dopa ethyl ester.

The MS spectra of Gd-DTPA-2LDEE displayed the correct peak of $M + H^+$ (MS 962.62), in accord with the molecular weight of Gd-DTPA-2LDEE (MW 961). The MS spectra of Gd-DTPA-2LDEE·H_2O showed M(·H_2O)+H^+ (MS 979.62), in accord with the molecular weight of Gd-DTPA-2LDEE·H_2O (MW 979). The MS spectra of ^{99m}Tc-DTPA-2LDEE displayed the correct peak of $M + H^+$ (MS 972.75), in accord with the molecular weight of ^{99m}Tc-DTPA-2LDEE (MW 971). MS spectra of ^{99m}Tc-DTPA-2LDEE·H_2O had the correct peak of M(·H_2O)+H^+ (MS 999.79), which is in accord with the molecular weight of ^{99m}Tc-DTPA-2LDEE·H_2O (MW 999). Thus, the results exhibited the formation of the ligand and metal complexes. However, good crystals of Gd-DTPA-2LDEE and ^{99m}Tc-DTPA-2LDEE were not easily obtained and their structures are not reported herein.

Recently, DTPA has been used as a ligand in nuclear medicine and MRI. The molecular structure of DTPA contains eight coordination sites, including three nitrogens and five carboxyl groups. Gd-DTPA and ^{99m}Tc-DTPA were prepared by chelation of DTPA with gadolinium and technetium-99m, respectively, using six coordination bonds to the DTPA molecular structure and one chelated water. Three coordination bonds are made by chelation of three nitrogens with gadolinium and technetium-99m, respectively, whilst three coordination bonds are produced by chelation of oxygens of three carboxyl groups. Therefore, Gd-DTPA derivatives possess the same coordination as Gd-DTPA. Gd-DTPA-2LDEE and

^{99m}Tc-DTPA-2LDEE were also prepared by chelation of DTPA-2LDEE with gadolinium and technetium-99m, respectively. Gd-DTPA-2LDEE and ^{99m}Tc-DTPA-2LDEE are expected to possess seven coordination bonds and the same coordination molecular structures as Gd-DTPA and ^{99m}Tc-DTPA, respectively. These results were proved by MS spectra of DTPA-2LDEE, Gd-DTPA-2LDEE, and ^{99m}Tc-DTPA-2LDEE.

3.2. *Relaxivity of gadolinium complex*

The gadolinium complex Gd-DTPA-2LDEE exhibited effective relaxation rates and improved the relaxivity per gadolinium due to a slowly tumbling system and an increase in rotational correlation time. According to equation (1), the curve was made by the value of

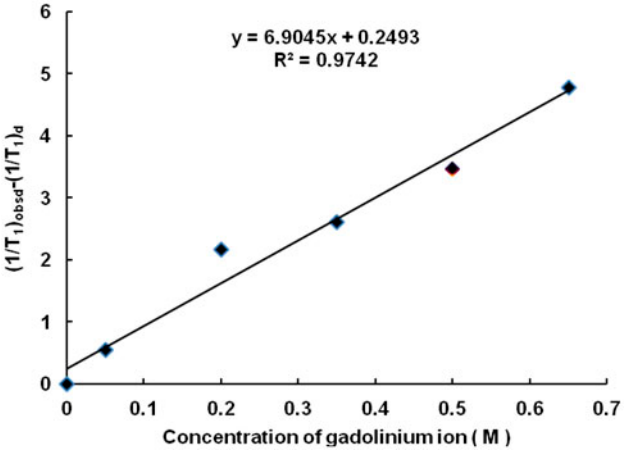


Figure 1. Relaxivity of gadolinium ions in water.

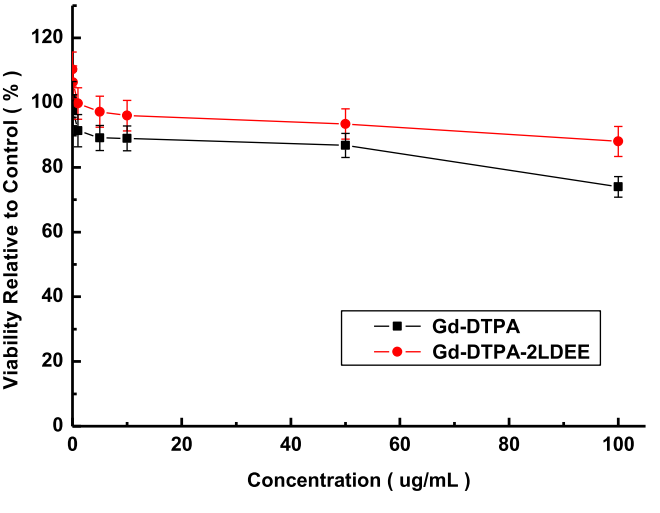


Figure 2. *In vitro* cytotoxicity assay of gadolinium complex to HepG-2 cells.

solvent relaxation rate $(1/T_1)_{obsd}-(1/T_1)_d$ as the *y*-axis vs. the concentration of gadolinium ions $[Gd^{3+}]$ as the *x*-axis (figure 1). The relaxivity of gadolinium chelate was enhanced when the concentration of gadolinium ion [M] increased. Then, the slope value of simulation linearity of 6.09 $mM^{-1} L s^{-1}$ represented the relaxivity r_1. Figure 1 illustrates that gadolinium complex Gd-DTPA-2LDEE possessed higher relaxation effectiveness than Gd-DTPA (3.75 $mM^{-1} L s^{-1}$) at the same condition.

3.3. In vitro *cytotoxicity assay*

The effect of gadolinium complex on HepG-2 cell growth and metabolism is shown in figure 2. At the concentration (10 μg/mL) of gadolinium complex in the growth medium, the viabilities of HepG-2 cells incubated with Gd-DTPA and Gd-DTPA-2LDEE were 88.97 and 96.0%, respectively, relative to control. At 100 μg/mL of gadolinium complex in the growth medium, the viabilities of HepG-2 cells incubated with Gd-DTPA and Gd-DTPA-2LDEE were 73.99 and 88.03%, respectively. Thus, Gd-DTPA-2LDEE possessed lower cytotoxicity to HepG-2 cells than Gd-DTPA.

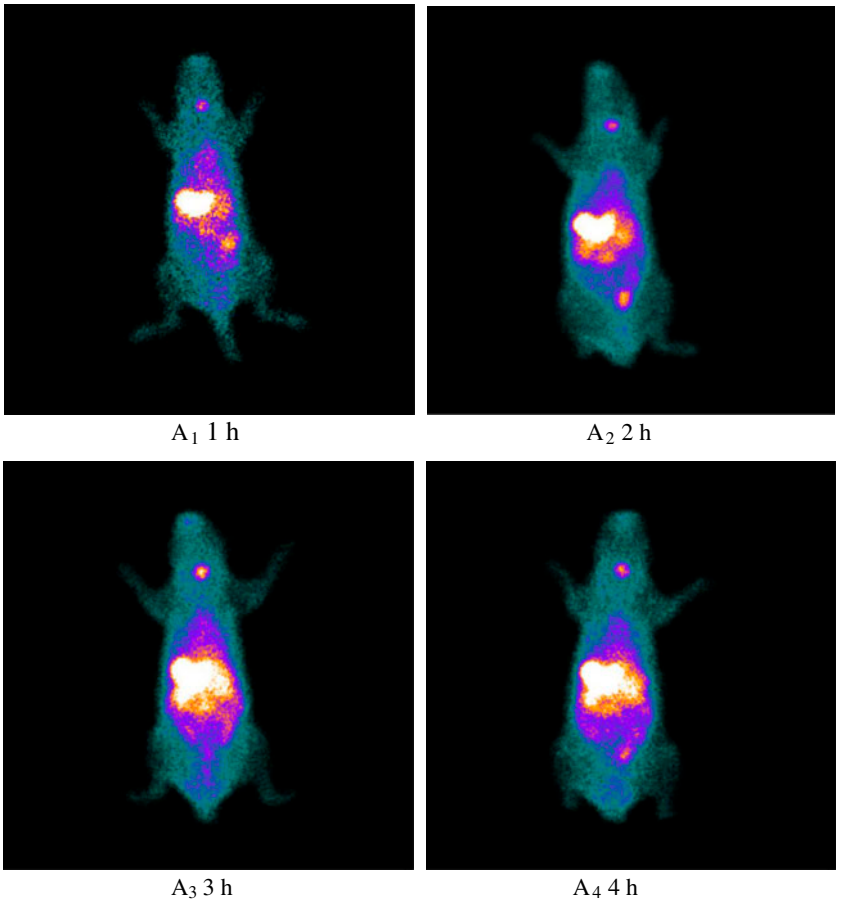


Figure 3. *In vivo* SPECT images of SD white rats having received injection of $Na^{99m}TcO_4$ (1.2 mCi) solution after 1, 2, 3, and 4 h, respectively.

3.4. *SPECT imaging*

The SPECT images of SD white rats having received injection with $Na^{99m}TcO_4$ (1.2 mCi), ^{99m}Tc-DTPA (0.25 mL, 1.2 mCi), or ^{99m}Tc-DTPA-2LDEE (0.35 mL, 1.2 mCi) *in vivo* at different times are shown in figures 3–5, respectively. Compared to rats having received injection with $Na^{99m}TcO_4$ (1.2 mCi), all signal intensities of the kidneys and bladder in rats injected with ^{99m}Tc-DTPA (0.25 mL, 1.2 mCi) and ^{99m}Tc-DTPA-2LDEE (0.35 mL, 1.2 mCi) were enhanced, the irradiated portions of the kidneys and bladder were brighter and their demarcations became clearer during the detection time, while those of the surrounding tissues, such as the muscle, bone, and stomach, showed little change.

Fifteen hours after injection with ^{99m}Tc-DTPA-2LDEE (1.2 mCi), the high SPECT signal enhancements of the kidneys in rats were still enhanced (figure 5). However, far less signal enhancements had been displayed in SPECT images of the kidneys in rats injected with ^{99m}Tc-DTPA (1.2 mCi) after 3 h (figure 4). Compared with ^{99m}Tc-DTPA, ^{99m}Tc-DTPA-2LDEE accumulated and remained in the kidneys significantly longer for 15 h, and

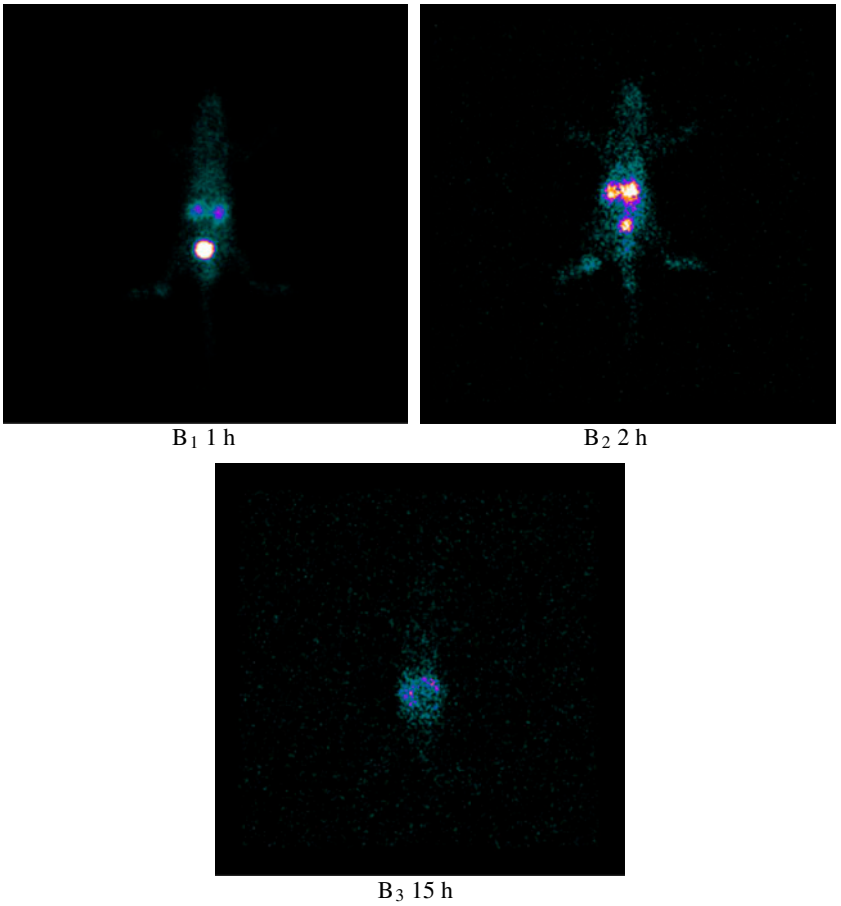


Figure 4. *In vivo* SPECT images of SD white rats having received injection of ^{99m}Tc-DTPA (0.25 mL, 1.2 mCi) solution after 1, 2, and 15 h, respectively.

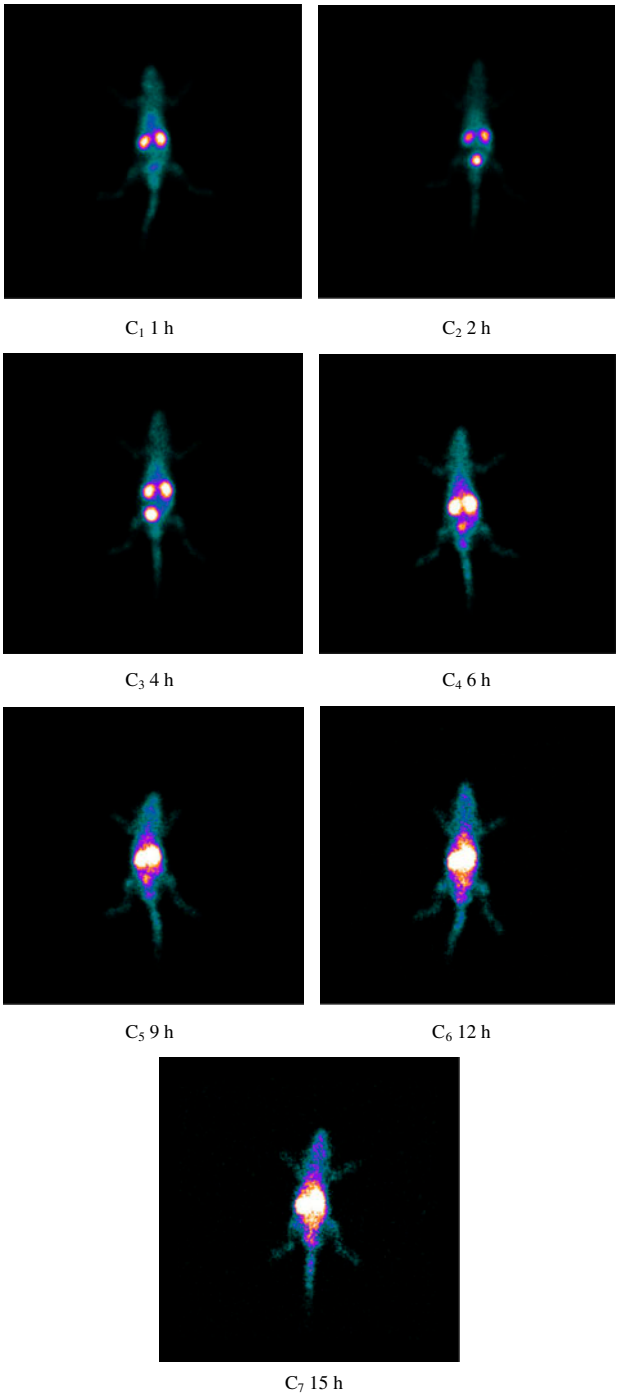


Figure 5. *In vivo* SPECT images of SD white rats having received injection of ^{99m}Tc-DTPA-2LDEE (0.35 mL, 1.2 mCi) solution after 1, 2, 4, 6, 9, 12, and 15 h, respectively.

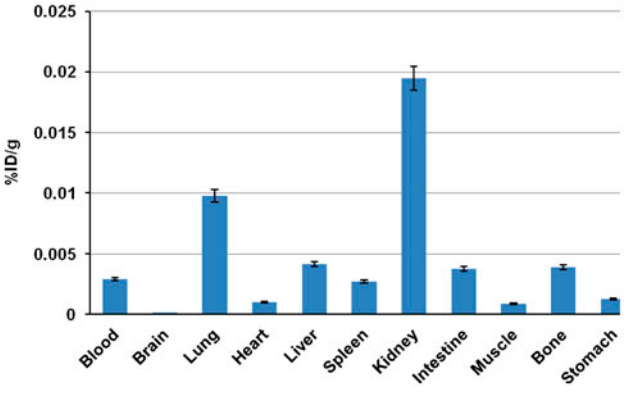


Figure 6. Organ distribution in SD white rats having received injection of ^{99m}Tc-DTPA-2LDEE (0.35 mL, 1.2 mCi) solution after 15 h.

possessed markedly higher SPECT signal enhancements of the kidneys in rats. These results indicated that ^{99m}Tc-DTPA-2LDEE had enhanced SPECT signals of the kidneys in rats and then can provide prolonged enhancement and longer duration time in the body than that of ^{99m}Tc-DTPA.

Figure 6 shows the organ and tissue distribution in SD white rats having received injection of ^{99m}Tc-DTPA-2LDEE (1.2 mCi) solution after 15 h. The kidneys and bladder had the higher percentage of injected dose per organ (%ID/g) than the other organs and tissues, demonstrating that ^{99m}Tc-DTPA-2LDEE and ^{99m}Tc-DTPA were mostly excreted by the kidneys. Interestingly, no obvious SPECT signal enhancements have been displayed in the brains during the detection times.

4. Conclusion

LDEE was incorporated to DTPA to produce DTPA-2LDEE. The corresponding neutral gadolinium complex Gd-DTPA-2LDEE possessed higher relaxation effectiveness and lower cytotoxicity to human hepatoma HepG-2 cells than that of Gd-DTPA. Moreover, technetium-99m-labeled complex ^{99m}Tc-DTPA-2LDEE had enhanced SPECT signal enhancements of the kidneys in rats and provided longer duration time than that of ^{99m}Tc-DTPA. These results indicated that ^{99m}Tc-DTPA-2LDEE can be selectively excreted by the kidneys and used as a potential signal-enhanced radioactive probe in SPECT.

Supplementary material

The 1H NMR spectra of DTPA-2LDEE, mass spectra of the ligand DTPA-2LDEE and complexes Gd-DTPA-2LDEE and 99mTc-DTPA-2LDEE have been deposited as a suplementary data (see online supplemental material at http://dx.doi.org/10.1080/00958972.2014.892591).

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