# Isostructural Re and $^{99m}$Tc Complexes of Gd-DTPA-Histidine for Dual-Modality MR/SPECT imaging agents

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## Introduction

Multi-modality imaging based on different detection techniques is a powerful approach to understand the complex phenomena of molecular events in *in-vivo* system, and can be more powered by multi-modality imaging probes. The combination of magnetic resonance imaging (MRI) and nuclear medicine imaging can be representative synergistic approach for providing anatomical and functional information together, because of the high resolution of MRI and the high detector sensitivity of radionuclide imaging, respectively. Single photon emission computed tomography (SPECT) is important nuclear medicine imaging tool in the evaluation of cardiovascular disease and tumor therapy responses, and the radionuclide of $^{99m}$Tc has been widely used in SPECT imaging. Therefore SPECT with $^{99m}$Tc can be a candidate for new hybrid imaging of MR/SPECT. Despite of the promising potential of MR/SPECT imaging, the approach for MR/SPECT imaging agent has rarely explored. In this study, we report the Gd-DTPA-histidine conjugated of $^{99m}$Tc complexes [GdLHis_$^{99m}$Tc] as MR/SPECT dual modality contrast agents. To overcome problems in hybrid imaging due to the huge difference (~$10^3$) of imaging sensitivity between MRI and SPECT, we suggest the mixed-usage of [GdLHis_$^{99m}$Tc] with Gd-DTPA-histidine conjugated of Re complexes [GdLHis_Re] that is an alternative substance (cold-form) having chemical characteristics equivalent with [GdLHis_$^{99m}$Tc].

## Material and Methods

A series of Re/$^{99m}$Tc complexes incorporating Gd-DTPA conjugate of histidine were prepared as illustrated in Scheme 1. Maldi-TOF mass spectra were obtained by using a Voyager-DETM STR Biospectrometry (Applied Biosystems Inc.). FT-IR spectra were carried out with a Bruker TENSOR spectrometer. $T_1$ measurements were carried out using an inversion recovery method with variable inversion time (TI) at 3 T (128 MHz). $T_1$ relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each TI value. Analytical HPLC was performed using Waters 2690 model and an Altlantis dC-18 column (3.0 × 150 mm, 5 $\mu$m). The mobile phase consisted of aqueous 0.1% TFA in acetonitrile (solvent A)/ 0.1% TFA in water (solvent B). The elution condition was 5% A over 25 min at flow rate of 0.43 mL/min. The reaction mixture was monitored by observing the UV profile at 254 nm for [GdLHis_Re] and radio-trace for [GdLHis_$^{99m}$Tc]. For *in vivo* study, the mice were anesthetized by 1.5% isoflurane in oxygen. MR images of mice were obtained pre- and post- [GdLHis_Re] (0.1 mmol Gd/kg) injection by tail vein with a 3 Tesla MR unit (Magnetom Trio Tim, Siemens, Erlangen, Germany). The imaging parameters for SE (spin echo) were as follows: repetition time (TR) = 752 ms; echo time (TE) = 13 ms; 10 mm field of view (FOV); 320×320 matrix size; 1 mm slice thickness; average = 4. Images were obtained during 240 min after injection. Serial single pin-hole SPECT images (32 min x 6 times) were obtained hour after injecting [GdLHis_$^{99m}$Tc] (1 mCi/50 g BW), using multi-modal SPECT/CT system (INVEON, Simens Medical Solutions). On the area of liver and kidney of serial images of MRI and SPECT, the elliptical shape of regions of interest (ROIs) were place to obtain the time courses of signal intensity and radioactivity, respectively.

## Results and Discussion

The formation of [GdLHis_Re] and [GdLHis_$^{99m}$Tc] were confirmed by analytical and spectroscopic techniques. The MALDI-TOF mass spectrum of [GdLHis_Re] exhibits the molecular peak at 1360 Da corresponding to $[M]^+$-$H_2O$. The binding of Re to the GdLHis can be confirmed by FT-IR. Namely, the C-O stretching band at 1995 and 1872 $cm^{-1}$ is typical for a Re(I)-tricarbonly complexes. The resultant relaxivity, $r_1$, of [GdLHis_Re] was quite high as compared with other MRI contrast agent currently in use. In the case of [GdLHis_Re], for instance, the $r_1$ relaxivity is 7.8 $m$$M^{-1}$$sec^{-1}$, which is twice as high as that of structurally related Magnevist® ($r_1$= 4.0 $m$$M^{-1}$$sec^{-1}$). This results indicated that [GdLHis_Re] have a potential ability to enhance $T_1$-weighted images. [GdLHis_$^{99m}$Tc] was obtained in high yield (more than 95%) with high radio chemical purity and characterized by comparing its HPLC profile with the profile of the corresponding [GdLHis_Re]. The retention time of [GdLHis_$^{99m}$Tc] (17.5 min) in the $\gamma$-HPLC matched that for the Re reference standard [GdLHis_Re](16.5 min), which was detected by UV. HPLC results showed that the chemistry of [GdLHis_$^{99m}$Tc] is similar to that of [GdLHis_Re]. The *in vivo* MR images of mice obtained with [GdLHis_Re] show the contrast enhancement liver and kidney. The enhancement of kidney indicates that elimination of [GdLHis_Re] take place mainly through glomerular filtration, as confirmed by Figure 1. An additional feature of [GdLHis_Re] is the ability of gallbladder enhancement as evidenced by both *in vivo* MR, indicates the bile excretion. The *in vivo* SPECT images with [GdLHis_$^{99m}$Tc] showed general agreement with the results of the [GdLHis_Re] contrast enhancement *in vivo* MR studies. High levels of activity were observed specially in the liver, kidney and gallbladder. It suggested that [GdLHis_Re] and [GdLHis_$^{99m}$Tc] exhibit similar biodistribution. By 24 h post injection, *in vivo* MR and SPECT images showed little activity in the body, corresponding to negligible amounts of circulating.

## Conclusions

A new hybrid $^{99m}$Tc labeled MR contrast agents could be made and be suitable to both MR and SPECT imaging. The identical characteristics of [GdLHis_$^{99m}$Tc] and [GdLHis_Re] showed the same bio-functional change of hybrid contrast agents, with the additional merit of improved MRI image quality. Therefore, the hybrid-usage of both [GdLHis_Re] and [GdLHis_$^{99m}$Tc] should be useful in the optimal acquisition of MR/SPECT imaging that is prerequisite for precise interpretation of bio-function.

**Scheme 1**
**Synthesis of GdLHis_Re/$^{99m}$Tc**

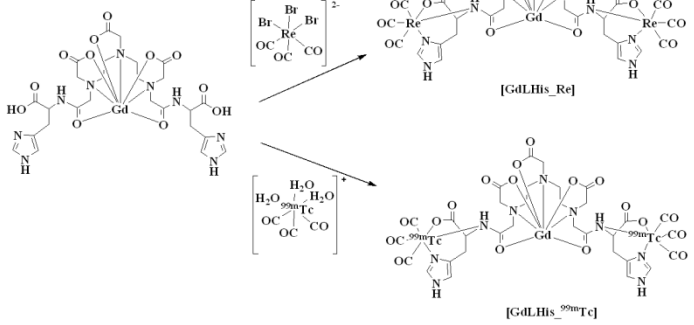


Figure 1. *In vivo* MR and SPECT images of mice