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GADOLINIUM LABELED PHARMACEUTICALS AS POTENTIAL MRI CONTRAST AGENTS FOR LIVER AND BILIARY TRACT

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SUMMARY

Three gadolinium-labeled compounds, potential nuclear magnetic resonance (NMR) imaging contrast agents for liver and biliary tract, were studied: 1) Gd-DISIDA, 2) Gd-DTPA-Liposomes, and 3) Gd-DTPA dihexadecylamide (Gd-diamide). In each case, "Carrier Added" Gd-153 with specific activity of about 5uCi/mg was used. Each labeled compound was evaluated in experimental animals. Gd-DISIDA proved unsatisfactory because of in vivo instability. Gd-DTPA-Liposomes demonstrated strong toxic effects probably due to pulmonary embolism when large amounts of this compound was administered intravenously. Gd-diamide showed good uptake in the hepatocytes with subsequent excretion into the biliary tract. Several rabbits were imaged in a 0.6T NMR imaging system before and after injection of Gd-diamide. Pulse sequences were chosen that would yield T1-weighted images and permit calculation of T1 relaxation times. This compound produced significant shortening of the T1 relaxation times of the liver and observable increase in intensity on the T1-weighted images. Gd-diamide shows promise as potential NMR contrast agent for liver and biliary tract imaging.

KEY WORDS: Biliary Tract Imaging, MRI Imaging, Gd-DISIDA, Gd-Liposome, Gd-DTPA diamide

INTRODUCTION

The development of nuclear magnetic resonance (NMR) imaging systems has provided a method for in vivo research in biological systems using a noninvasive technique. Contrast in NMR images is dependent upon three parameters: proton concentration or density, spin-lattice relaxation time (T1) and spin-spin relaxation time (T2). Ions and molecules containing unpaired electrons demonstrate a paramagnetic behavior, which causes a change in the relaxation times of tissues containing these ions. Therefore one method for enhancing contrast in nuclear magnetic resonance imaging is the use of paramagnetic ions (1). One of the most

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interesting ions with strong paramagnetic properties for use as a contrast agent in NMR is gadolinium (2). However, unless gadolinium is chelated to strong chelating agents such as DTPA, or EDTA, this ion is too toxic to be considered for intravenous use in humans. Gadolinium labeled DTPA has been used extensively as a contrast agent for brain and kidney imaging (2). The purpose of this investigation was to find a gadolinium complex suitable for biliary tract imaging. Gadolinium labeled DISIDA and Gadolinium labeled liposomes were examined first, since technetium analogues of these pharmaceuticals are well known liver imaging agents (3,4) in Nuclear Medicine. Gd-diamide was examined next since its technetium analogue also is reported to be a liver imaging agent (5).

Methods and Results

All non-radioactive materials were obtained from Aldrich chemical company (Milwaukee, WI). Gd-153 labeled gadolinium chloride was obtained from New England Nuclear (Boston, MA). Nuclear Medicine images were obtained by using a MDEX scintillation camera and

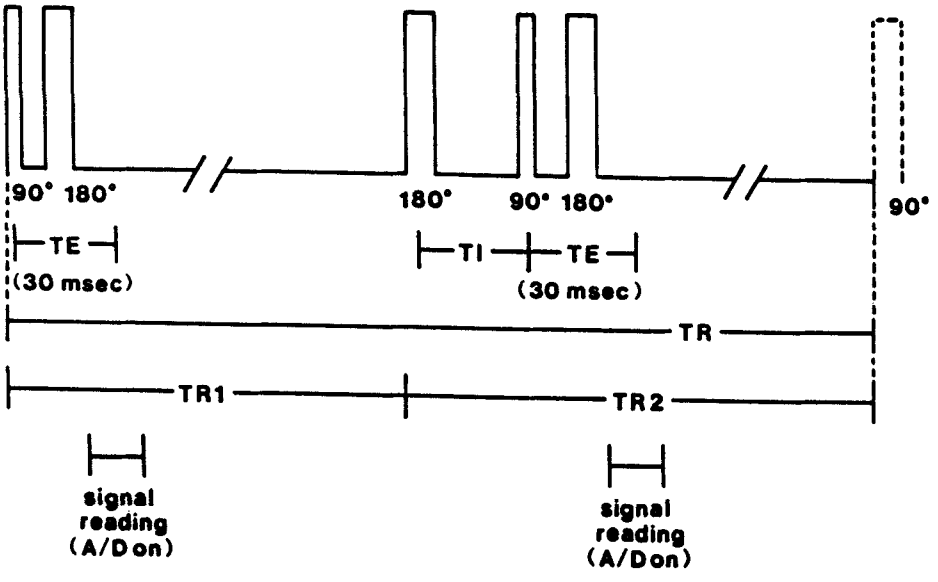

Figure 1 OTl pulse sequence including spin echo 30 and inversion spin echo 30.

collecting 50,000 counts per image. The NMR imaging procedure used to calculate the T1 values was accomplished using a manufacturer (Technicare) supplied T1 acquisition algorithm which includes a combination of a spin echo technique with TE = 30 msec, and TR = 1137 msec, and an inversion spin echo technique with TE = 30 msec, TR = 1500 msec, and TI = 364 msec. The "OT1" (observed T1) software pulse sequence diagram is seen in figure 1. Both pulse sequences are performed sequentially for each change in the phase encoding gradient. The imaging technique was started using T1 weighted spin echo sequence with TR = 300 msec and TE = 40 msec to locate the liver and then a single slice acquisition was performed using the OT1 combination sequence for measuring T1 relaxation time.

Preparation

1. Gd-153 Labeled DISIDA (3)

Gadolinium oxide (36 mg, 0.1 mmole) was disolved in minimum volume of concentrated hydrochloric acid (about 100 microliter). This was diluted with distilled water (1.0 ml) and mixed with about 150uCi of Gd-153 gadolinium chloride. This solution was added dropwise with vigorous stirring to a solution of N-(2,6 Diisopropylphenylcarbamoylmethyl) iminodiacetic acid (DISIDA) (140 mg, 0.4 mmole) dissolved in 2.5N phosphate buffer, (3.0 ml), pH = 7.0. The mixture was then spotted on Whatman No. 1 paper for development in concentrated acetic acid eluant. Radioactivity bound to DISIDA migrates to the solvent front whereas gadolinium chloride remains at the origin. The percentage of the radioactivity in the symmetric peak at the solvent front with respect to that in the entire chromatogram was more than 95%.

2. Gd-153 labeled gadolinium Liposomes

Gd-153 labeled gadolinium liposomes were prepared by modification of the reported method of Hnatowich et al (4). 1.5 g of phosphatidylcholine dipalmitoyl (lecithin), and 115 mg of

cholesterol were mixed well before "Carrier Added" Gd-153-diamide (165 mg, 150uCi) in 10 ml of saline was added to the mixture, The final mixture was vortexed and sonicated to produce a uniformed gel. A sample of this mixture was then passed through 0.22 micron filter to check for purity. Radioactivity bound to liposomes will not pass through this filter whereas all soluble forms of the radioactivity will. The percentage of the radioactivity which stayed in the filter with respect to that in the entire sample was more than 95%. The particle size of these liposomes were measured by using a microscope and were observed to range between 0.2 - 0.3 microns.

3. Gd-153 labeled gadolinium DTPA dihexadecylamide

DTPA Dihexadecylamide (Gd-diamide) was prepared using the method described by Hnatowich, et al. (4). This involved the preparation of the bicyclic anhydride of DTPA (5) and its reaction in dimethylformamide with two equivalent amounts of hexadecylamine. This product was identified by ^{1}H NMR spectroscopy in trifluoroacetic acid, and elemental analysis (Galbraith Laboratory, Knoxville, TN). A similar method as described for labeling DISIDA was used to label this product with Gd-153. The DTPA diamide (115 mg, 0.14 mmole) was labeled using Gd-153 labeled gadolinium chloride (50 mg, 150uCi).

Animal biodistribution studies

1. Gd-153 labeled DISIDA

Under sodium pentabarbital anesthesia, three groups of five female Sprague Dawley rats weighing about 150 g were injected via their tail vein with 5-6uCi of the "Carrier Added" Gd-153 labeled gadolinium DISIDA. These animals were sacrificed at 10, 30, and 180 min. after injection. Tissue samples were counted in a sodium iodide well counter and compared to a reference sample.

Table I

The result of the biodistribution study of "Carrier Added" Gd-153 labeled gadolinium DISIDA in rats normalized to 150 g. Values are expressed as %dose/gr tissue.

	Time Post Injection		
Tissue	10 Min.	30 Min.	180 Min.
Blood	3.27(0.34)*	3.56(0.31)	3.36(0.21)
Liver	1.75(0.25)	1.82(0.16)	2.08(0.17)
Spleen	0.98(0.21)	0.75(0.08)	0.82(0.07)
Kidney	1.46(0.11)	1.82(0.27)	1.70(0.14)
Heart	0.56(0.23)	0.35(0.09)	0.45(0.03)

*Standard Deviation

The results in Table I suggests breakdown of the labeled product in vivo since the radioactivity remains in vascular system. To confirm this a sample of Gd-DISIDA was mixed with equal volume of HSA and incubated for 5 minutes. This mixture was then spotted on Whatman paper No. 1 for development in concentrated acetic acid as eluant. This test revealed only one peak at the origin of the paper which also suggests breakdown of Gd-DISIDA.

2. Gd-153 labeled Liposomes

Under sodium pentabarbital anesthesia two rabbits weighing about 5 kg were injected with about 0.5g of the previously prepared Gd-153 labeled liposomes intravenously. These rabbits died while the injection was in progress probably due to pulmonary embolism. Difficulties with injecting this product prevented further investigation.

3. Gd-153 labeled DTPA dihexadecylamide

Under sodium pentabarbital anesthesia, five female Sprague Dawley rats weighing about 200 g were injected via the tail vein with 5-6uCi of the "Carrier Added" Gd-153-diamide. These animals were sacrificed 10 min after the injection. Tissue samples were counted in a sodium iodide well counter and compared to a

reference sample. The results shown in Table II demonstrate intense hepatic uptake.

Table II

The results of the biodistribution study of "Carrier Added" Gd-153 labeled gadolinium DTPA Dihexadecylamide in five rats, normalized to 150 g. Values are expressed as %d/gr tissue, 10 min after injection.

Liver	3.17 (0.23)*
Heart	0.29 (0.07)
Kidney	0.51 (0.12)
Blood	0.73 (0.18)
Spleen	0.54 (0.15)

*Standard Deviation

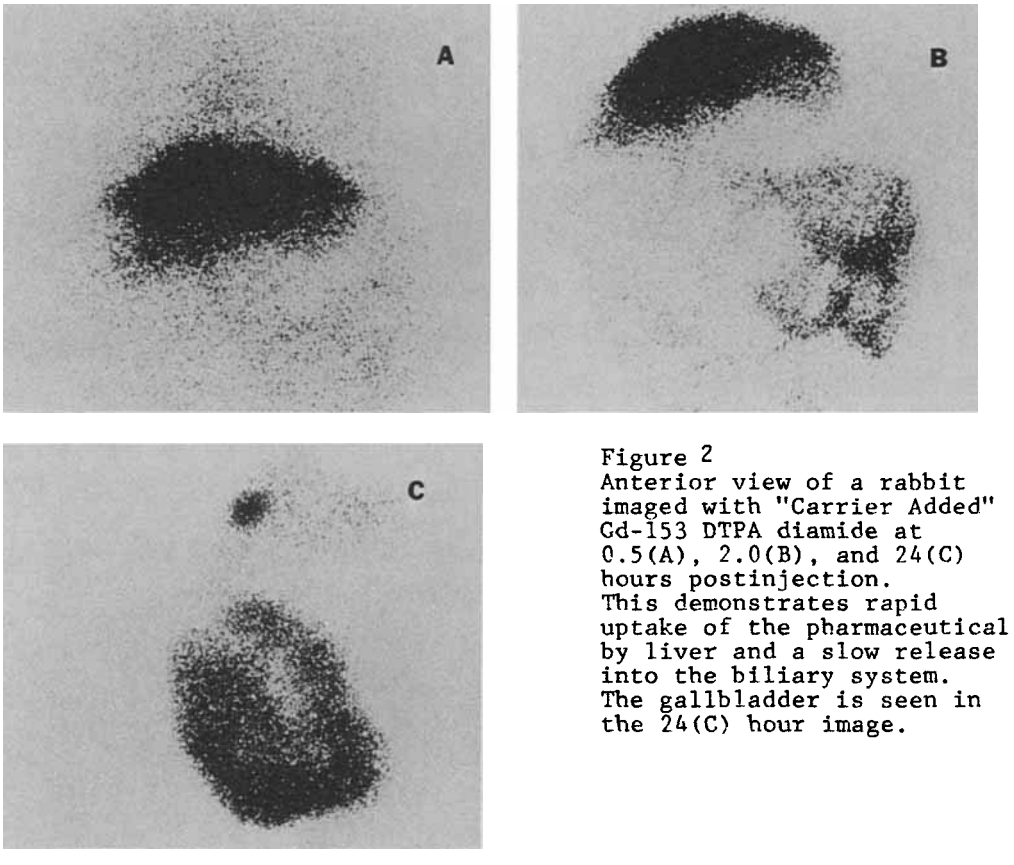

Figure 2
Anterior view of a rabbit imaged with "Carrier Added" Gd-153 DTPA diamide at 0.5(A), 2.0(B), and 24(C) hours postinjection. This demonstrates rapid uptake of the pharmaceutical by liver and a slow release into the biliary system. The gallbladder is seen in the 24(C) hour image.

Table II demonstrates intense hepatic uptake. Under sodium pentabarbital anesthesia, three rabbits also were injected with about 50uCi of the above product. Gamma camera images of these rabbits were recorded at 10 min, 2.0 hr., and 24 hrs. after injection (figure 2). These rabbits also were imaged with a 0.6T NMR imaging system before and after injection (figure 3). T1 relaxation times of three regions of the liver of each rabbit were measured before and after injection and are shown in Table III. The T1 relaxation time of normal liver was observed to be shortened by about 60% after administration of the pharmaceutical. These rabbits did not show any toxicity effect from the pharmaceutical a month after injection.

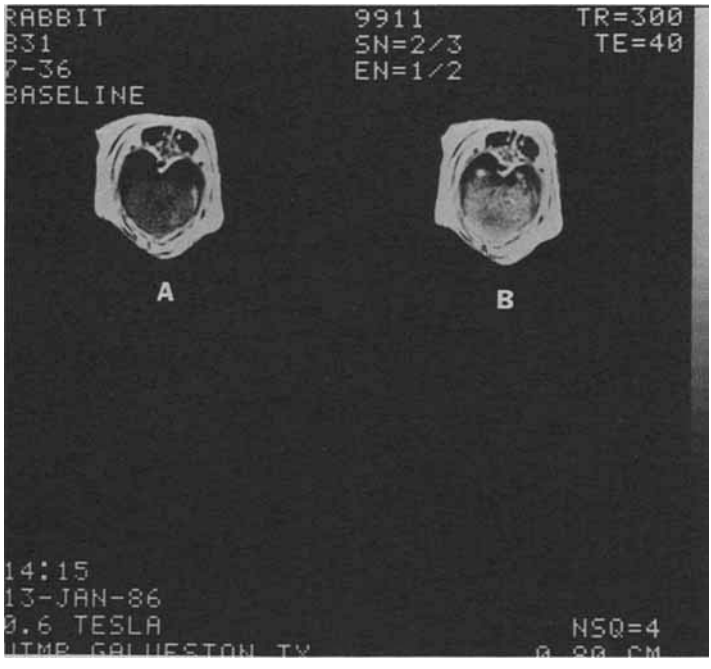

Figure 3 NMR images of a rabbit using a 0.6T NMR imaging system before (A) and after (B) injection with "Carrier Added" Gd-153 DTPA diamide

Table III

T1 relaxation time (msec) of three rabbits imaged with the Gd-DTPAdiamide, and 0.6T NMR imaging.

		Pre. Inject.	Avg.±SD	Post-Inject.	Avg.±SD	Percent Shorten.
Rabbit 1						
Liver:	Region A	370	359	161	148	
	Region B	378	±26	147	±12	59%
	Region C	330		136		
Rabbit 2						
Liver:	Region A	305	369	147	147	
	Region B	379	±59	140	±9	60%
	Region C	421		158		
Rabbit 3						
Liver:	Region A	404	432	158	158	
	Region B	455	±6	120	±38	63%
	Region C	438		197		

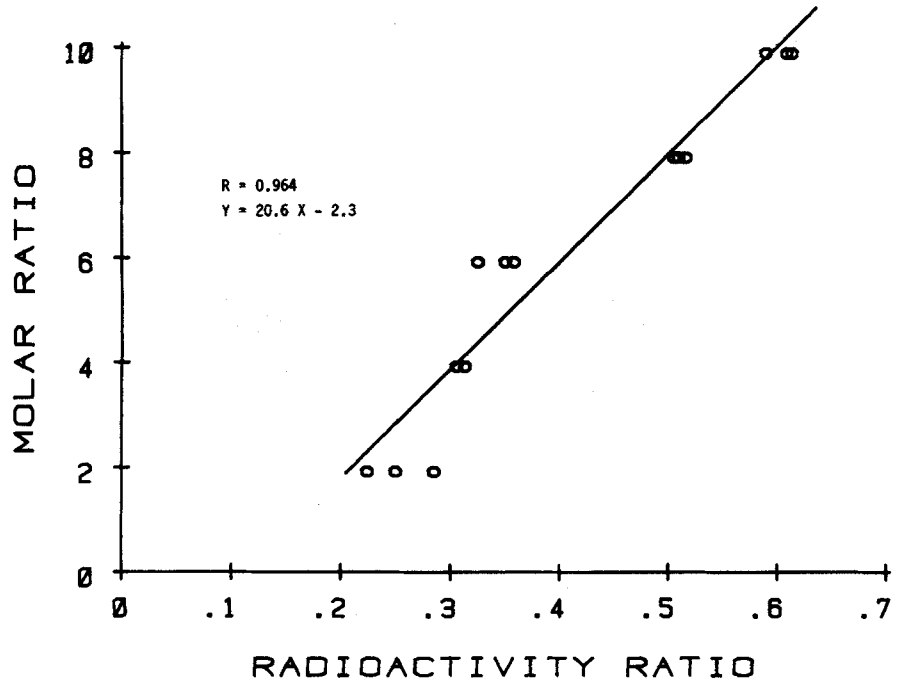

Figure 4 Effect of DTPA diamide/DTPA molar radio (ordinate) on DTPA diamide/DTPA radioactivity ratio (abscissa).

Relative Formation Constant Measurement

A modified method (6) was used to measure the relative formation constant of DTPA diamide with Gadolinium. Competitive studies for Gd-153 were performed by preparing solutions containing different concentrations of DTPA diamide and DTPA in the range of 0-25 mg/ml in phosphate buffer. Approximately 5.0μCi of Gd-153 was added as the chloride to each solution. Each solution was then carefully neutralized to pH = 7.0 and allowed to stand at room temperature for two hours before it was analyzed by paper chromatography using Whatman No. 1 paper and 0.1 N acetate buffer at pH = 7.0. The labeled diamide remains near the origin while labeled DTPA migrates to the solvent front. Each chromatogram was cut in half and each half counted separately in a NaI (Tl) well counter. Reanalysis of several samples at 24 hrs. after this study showed that equilibrium was established.

Figure 4 shows the effect of the DTPA diamide to DTPA molar ratio (ordinate), and the DTPA diamide to DTPA radioactivity ratio (abscissa). A least squares regression analysis provides a slope of about 20 with a correlation coefficient of 0.964. This suggests that the relative formation constant of DTPA diamide is about 20 times less than that of DTPA with gadolinium.

DISCUSSION

Gadolinium complexed with strong chelating agents such as DTPA has shown no short-term toxicity in imaging with NMR. These compounds are distributed within the vascular system and excreted rapidly unchanged through the urinary system. This search for a suitable gadolinium complex for liver and biliary tract imaging began with evaluation of gadolinium DISIDA

(2,6 Diisopropylacetanilideiminodiacetic acid), since the technetium analog of this pharmaceutical (Tc-DISIDA) is a well known liver imaging agent in Nuclear Medicine laboratories. This

complex was formed easily, but rat biodistribution studies demonstrated no accumulation of radioactivity in the liver. The radioactivity found in liver tissue in Table I is due to vascularity of liver and not because of hepatic uptake. Indeed most of the gadolinium was found in the blood and suggested in vivo instability of the complex. This is possible since DISIDA is a weaker chelating agent than DTPA. The instability of Gd-DISIDA was confirmed by paper chromatography.

Another well known technetium labeled liver imaging agent in Nuclear Medicine laboratories is Tc-liposomes. Therefore gadolinium labeled liposomes was evaluated next, but required about 0.5 g of this pharmaceutical to be administered in order to reach a hepatic concentration of approximately 0.1 millimolar Gd-DTPA, which resulted (7) in maximal increase in signal intensity in magnetic resonance imaging. The particle size of the labeled liposomes before administration was checked very carefully. Although a report in the literature (8) suggest that this test is successful, the two experimental animals tested in this experiment could not tolerate the liposomes and died. Therefore no further attempts were tried in this experiment.

The third investigational pharmaceutical was gadolinium complexed with DTPA dihexadecylamide. This pharmaceutical was evaluated because DTPA dihexadecylamide labeled with Tc-99m has been reported (4,5) as a suitable biliary tract imaging agent in Nuclear Medicine. Rat biodistribution study using "Carrier Added" Gd-153 DTPA diamide revealed rapid blood clearance and hepatic uptake in only a few minutes. The gamma camera images (figure 2) of a rabbit imaged with this compound also showed very rapid liver uptake. These images demonstrated that this compound remains in the liver for a period of time adequate for NMR imaging. Figure (3) illustrates an NMR image of a rabbit before and after intravenous injection of this compound and reveals increased

contrast in the area of the liver. Table III shows an average 60% reduction in the T1 relaxation time observed from three rabbits liver measurements before and after intravenous injection of this pharmaceutical. The radiofrequency (RF) timing parameters used in the NMR images were optimized for normal liver. Because the T1 shortening was so dramatic, the observed T1 values after injection may be slightly overestimated. The result would imply on even greater difference in T1 relaxation times pre- and post-injection. The gadolinium labeled DTPA diamide demonstrated a marked T1 shortening and shows great promise as a liver and biliary tract NMR contrast imaging agent.

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