Comparison of the Biodistribution of Manganese-54 DTPA and Gadolinium-153 DTPA in Dogs

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The biodistribution of [^{54}Mn]DTPA and [^{153}Gd]DTPA dimeglumine were investigated and compared following i.v. administration to fasting anesthetized dogs. Unlike most previously reported metal ion-DTPA complexes, [^{54}Mn]DTPA showed high uptakes in several organs including the liver, bile, pancreas, bowel, and kidney. This uptake was independent of the pH of the injected solution. Accumulation in these organs suggests a potential role for [Mn]DTPA as a paramagnetic contrast agent for NMR imaging. With the exception of the kidneys, [^{153}Gd]DTPA showed no evidence of tissue specific uptake over the course of 4 hr, consistent with it being an extracellular ion that is cleared by glomerular filtration.

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Although nuclear magnetic resonance (NMR) imaging is capable of exquisitely defining anatomy of the head, neck, pelvis, and extremities, its ability to consistently differentiate normal from abnormal tissue based on relative signal intensity is currently being refined (*1*). This is especially true in the abdomen where motion artifacts make imaging more difficult. Since healthy and pathologic tissues may have very similar relaxation constants, several approaches including improved pulse sequences, respiratory gating, and paramagnetic contrast agents are being investigated to help improve this differentiation. Paramagnetic substances reduce spin-lattice (T1) and spin-spin (T2) relaxation times and therefore change the NMR signal strength. In addition to improving lesion detectability, contrast agents should improve the ability of NMR imaging devices to assess organ physiology, rather than just anatomy. Currently gadolinium-diethylenetriaminepentaacetic acid ([Gd]DTPA) is receiving considerable attention as a relatively nontoxic, paramagnetic agent to enhance relaxation rates (*2–5*). However, [Gd]DTPA is an extracellular ion and, with the exception of the kidneys, no organ uptake of [Gd]DTPA has been demonstrated. For this reason many other agents are also currently under investigation (*5–8*).

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We have previously reported preliminary results of the biodistribution of manganese-54 (^{54}Mn)DTPA in dogs (*9*). We found that chelating the ^{54}Mn with DTPA greatly increased its urinary excretion while maintaining tissue specific uptake in liver, pancreas, bile, bowel, and kidneys. NMR images obtained following the injection of [Mn]DTPA showed dramatic negative enhancement of the gallbladder and to a lesser degree the liver. The kidneys were positively enhanced. These initial preliminary results prompted us to investigate in more detail the biodistribution of [^{54}Mn]DTPA and to compare it directly with [^{153}Gd]DTPA.

Despite the large number of manuscripts published about [Gd]DTPA, we were not able to locate any reports of its actual time dependent tissue distribution using a radiotracer technique. This technique is much simpler than assaying tissue samples for either the indirect effect of Gd on T1 or the concentration of metal determined by atomic absorption spectrometry or neutron activation analysis. One previous investigation only examined the distribution of [Gd]DTPA for 15 min postinjection (*5*). Distribution was determined indirectly through effects on T1 and T2 and then rank correlating these results with the distribution of technetium-99m- (^{99m}Tc) DTPA. Our experience with [Mn] DTPA showed that it may take several hours for useful organ uptake to become apparent (*9*). The purpose of this investigation was to further examine the biodistribution of [^{54}Mn]DTPA and compare it with the distribution of [^{153}Gd]DTPA in the same experimental ani-

mal model. Since the concentration of free ligand in these chelation reactions is dependent on the pH of the solution, we also examined whether varying the pH of the solution would alter the biodistribution of [^{54}Mn]DTPA.

MATERIALS AND METHODS

Preparation of DTPA Solutions

DTPA as the free acid was obtained.* One-tenth of a mole of this chemical was suspended in 50 cc of sterile water, followed by sequential addition of 0.1 moles each of calcium hydroxide and NaOH. The solution was stirred constantly, NaOH (1 *M*) was then added until the pH was 7.5. Next, 0.1 moles of $MnCl_2$ was added and the pH readjusted to the desired value (4.9, 5.9, 6.9, or 7.4) using 1 *M* NaOH and/or HCl. The solution was then brought to a final volume of 100 cc or 200 cc (1 or 0.5 *M*, respectively) and passed through a 0.45 micron millipore filter into sterile evacuated vials. Solutions were refrigerated for a period not exceeding 6 mo.

[Manganese-54]$MnCl_2$ (93.2 GBq/mMol) was obtained.† Just prior to its being used 100 μCi (3.7 MBq) of [^{54}Mn]$MnCl_2$ was added to the required amount of unlabeled material (0.1 m*M*/kg). A standard was then prepared for all injected solutions by withdrawing a 50 μl aliquot and diluting it to 25 cc. Duplicate 1-cc aliquots of the standard were placed in a gamma well counter and assayed. The [^{153}Gd]DTPA dimeglumine (33.3 GBq/mMol) and [Gd]DTPA dimeglumine for this investigation were supplied through the courtesy of Schering-Berlex, Berlin. Both of the solutions had a Gd/DTPA stoichiometry of 1 to 1, i.e., the same as for the [Mn]DTPA solutions. A dose of 0.1 m*M*/kg of unlabeled material was withdrawn after which ~100 mCi (3.7 MBq) of the labeled material was added. The solution was thoroughly mixed and an aliquot was withdrawn to prepare a standard solution as for [^{54}Mn]DTPA.

Surgical Technique

Over the course of several weeks, 12 fasting mongrel dogs were anesthetized with 25 mg/kg sodium pentobarbital intravenously. Two i.v. lines were inserted; one was used for injection purposes and fluid maintenance while the other was used to withdraw blood samples. A large midline abdominal incision was made after which a large bore pigtail catheter was inserted directly into the bladder and tied in place in such a manner as to minimize bladder volume. After surgery, the animal was kept in a Plexiglas semi-cylinder designed and built for this purpose. Throughout the experiment the animal was covered with incontinent pads and sheets to conserve body heat. An endotracheal tube provided a patent airway. The wound was closed with clips except when biopsy samples were withdrawn. The organs were manipulated gently and as little as possible. Clamps were placed on the organ of interest, isolating a small portion of the tissue, then forceps and scissors were used to remove one sample of the organ at each time point. The clamp was left in place to provide hemostasis. Subsequent biopsies avoided previously sampled or manipulated areas.

Biodistribution Studies

The animals received 0.1 mmol/kg of labeled [Mn]DTPA or [Gd]DTPA by bolus i.v. injection. The precise quantity of material injected was determined from assays of the standard solution and the exact volume injected. At 3, 15, 30, and 60 min and at 2 and 4 hr postinjection a sample of blood, pancreas, liver, and bowel were obtained as outlined in the surgical technique. All specimens were placed in preweighed gamma counting vials, weighed, and then assayed for radioactivity in a gamma counter. Urine was collected for assessment of the volume excreted. At 4 hr postinjection, the animal was killed by the injection of a 20-cc bolus of saturated KCl i.v. The liver, pancreas, kidneys, heart, and spleen were removed and weighed. A sample of these organs, as well as skeletal muscle and fat were assayed for radioactivity as described above. Three animals were injected with both [^{54}Mn]DTPA and [^{153}Gd]DTPA. To correct for Compton scatter (spill) from the higher energy ^{54}Mn emission, spectra were obtained for each of the radionuclides and separate windows were defined for each. The percent spill from the ^{54}Mn window into the ^{153}Gd window was calculated and this correction factor was applied to the experimental samples. All samples were also background corrected. These data were used to determine the percent injected dose/g × kg body weight (% i.d. g × kg BW), or percent of injected dose per organ. The former percentage representation was used to normalize the percent injected dose per gram to the animals total weight. Statistical calculations were performed using the Student's t-test.

RESULTS

Effect of pH on the Biodistribution of [Mn]DTPA in Dogs

We varied the pH of the [^{54}Mn]DTPA solution and injected it into a series of nine dogs. The pH was 4.9 for two dogs, 5.4 for three dogs, 6.9 for three dogs, and 7.4 for one dog. No consistent effect of the pH of the injected solution was observed in terms of any of the organs' uptake with time or on the final concentration of tracer in the organs studied. Samples results from these experiments are shown in Figures 1A and 1B. It is readily apparent that the percent of the injected dose in the liver is independent of the pH of the injected solution. Similarly the urinary excretion shows no significant change as a function of pH. Similar results were observed for all of the other organs studied.

Since the pH of the injected solution did not have an effect on the biodistribution of [^{54}Mn]DTPA, these results were pooled into one group in order to improve the statistical accuracy of the experiments. In Figure 2 the concentration of [^{54}Mn] (administered as [^{54}Mn]DTPA) in liver, bowel, pancreas, and blood are shown as a function of time. Time-dependent tissue uptake of the tracer is noted for the former three organs. The concentrations in the other organs at 4 hr postinjection are shown in Figure 3. There was no significant difference between the concentrations of ^{54}Mn in the kidney cortex and medulla. The myocardium accumulated a relatively low concentration of ^{54}Mn. The percent of [^{54}Mn]DTPA accumulating in each organ is shown in

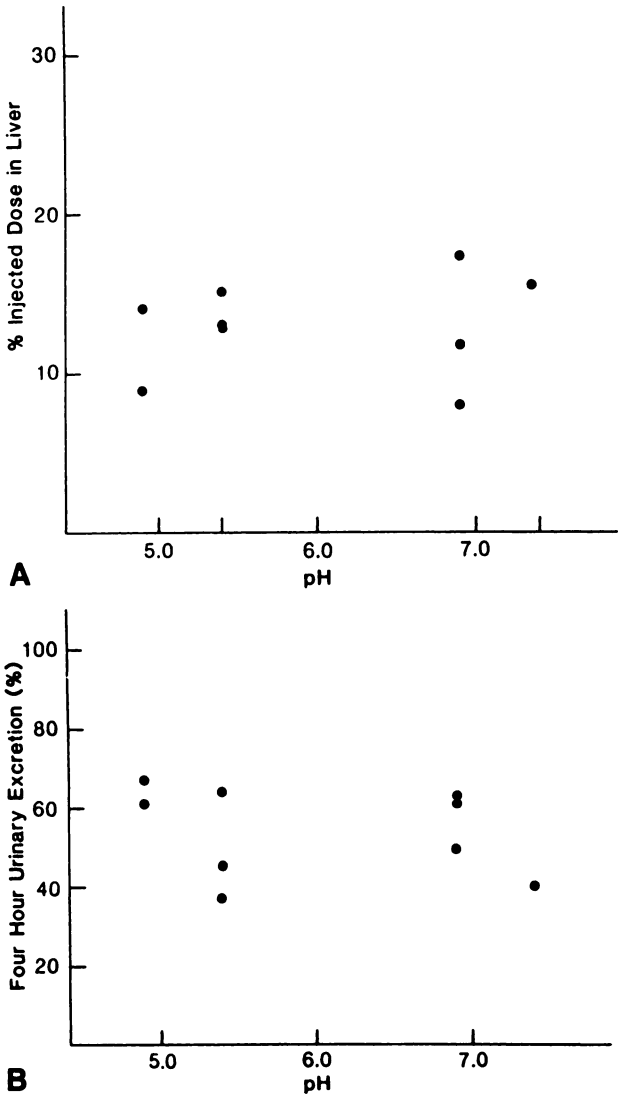

FIGURE 1
A: The percent injected dose in liver at 4 hr postinjection is plotted versus the pH of the injected [^{54}Mn]DTPA solution. Each data point represents one dog. B: The percent 4-hr urinary excretion is plotted versus pH of the [^{54}Mn] DTPA solution. Each data point represents one dog.

Table 1. The high urinary excretion of [Mn]DTPA is of interest in that unbound Mn is not cleared by the kidneys (*10*).

Comparison of [^{54}Mn]DTPA and [^{153}Gd]DTPA in Dogs

In Figure 4 the concentrations of ^{153}Gd administered as the DTPA chelate are shown as a function of time for liver, blood, and pancreas. Except for the kidney, none of the organs examined showed any evidence of tissue uptake. Their concentrations declined to relatively low levels that paralleled the fall in blood concentrations. The renal accumulation is presumably due to the fact that [Gd]DTPA is cleared by glomerular filtration. The concentrations of tracer in the liver are approximately ten times higher for [^{54}Mn]DTPA than for

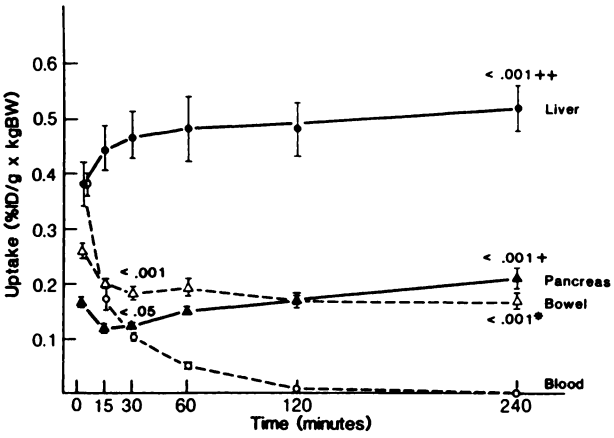

FIGURE 2
The uptake of ^{54}Mn (injected as [^{54}Mn]DTPA) in the liver, pancreas, bowel, and blood of fasting anesthetized dogs is shown as a function of time. Numbers refer to the p value of a Student's T-test of each point compared with blood at the same time. All of the liver values beyond 3 min were significant at the $p < 0.001$ level. (+): The pancreas accumulation had a $p < 0.05$ comparing uptake at 240 min with 3 min, and $p < 0.001$ comparing 240 min with 30 min. (++): The liver accumulation had a $p < 0.05$ comparing uptake at 240 min with 3 min. (*): The bowel concentration had a $p < 0.01$ comparing uptake at 240 min with 3 min.

[^{153}Gd]DTPA and the concentrations in the pancreas are approximately twenty-fold higher for the manganese complex. The urinary excretion of the [^{153}Gd]DTPA was considerably higher than [^{54}Mn]DTPA with 92% of the injected Gd dose having been excreted by 4 hr compared to 56% for [Mn]DTPA.

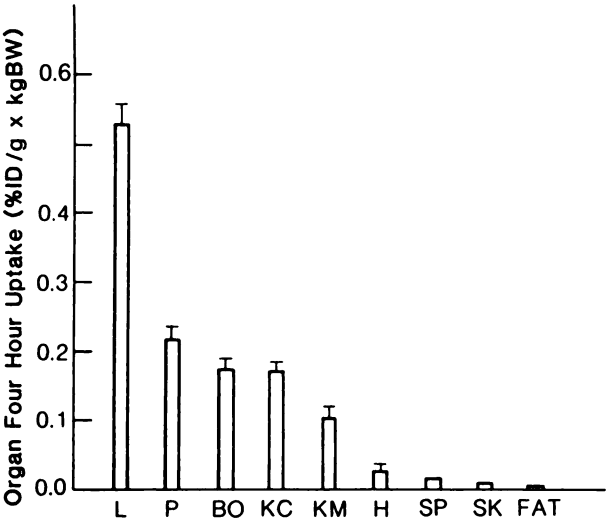

FIGURE 3
The 4-hr uptake of ^{54}Mn (injected as [^{54}Mn]DTPA) is shown for liver (L), pancreas (P), bowel (BO), kidney cortex (KC), kidney medulla (KM), heart (H), spleen (SP), skeletal muscle (SK), and fat. Results are expressed as mean ± s.e.m. of either nine dogs for L, P, and BO or six dogs for KC, KM, H, SP, SK, and fat.

TABLE 1
Percent Injected [^{54}MN]DTPA per Organ at 4 hr

Urine	55.9 ± 3.5
Liver	12.6 ± 1.0
Pancreas	0.52 ± 0.08
Gallbladder bile*	3.2 ± 0.64
Kidney*	0.66 ± 0.08
Spleen*	0.11 ± 0.02
Heart*	0.20 ± 0.002
Blood†	0.33 ± 0.06

*n = 6 dogs, otherwise n = 9 dogs. Results expressed as mean ± s.e.m.
† Assuming a blood volume of 2 l.

DISCUSSION

The observation of organ concentration of ^{54}Mn administered as [^{54}Mn]DTPA to fasting anesthetized dogs, is interesting. Many other DTPA-metal complexes including technetium-DTPA, ytterbium-DTPA, yttrium-DTPA, indium-DTPA, and lanthanum-DTPA are distributed essentially exclusively in the extracellular space and excreted by glomerular filtration (*11*). We cannot state with certainty at this time whether the tissue activity represents Mn++ or [Mn]DTPA. We have previously discussed the probable mechanism of uptake (*9*). Briefly, it is known that several tissues have high affinity binding sites for the manganese ion (*12–15*). These sites are likely competing with DTPA and other endogenous ligands for the manganese ion. We cannot exclude the possibility that some of the material is taken up as [Mn]DTPA. However, based on the observations that other DTPA complexes are distributed essentially exclusively in the extracellular spaces, are excreted solely by glomerular filtration, and show no tissue specific uptake, this mechanism would be unlikely to predominate (*11*). Our results with [Gd]DTPA confirm the indirect measurements of others (*5*) that show no evidence for uptake in normal tissue other than the kidneys. However, our observations were extended over a period of 4 hr rather than 15 min.

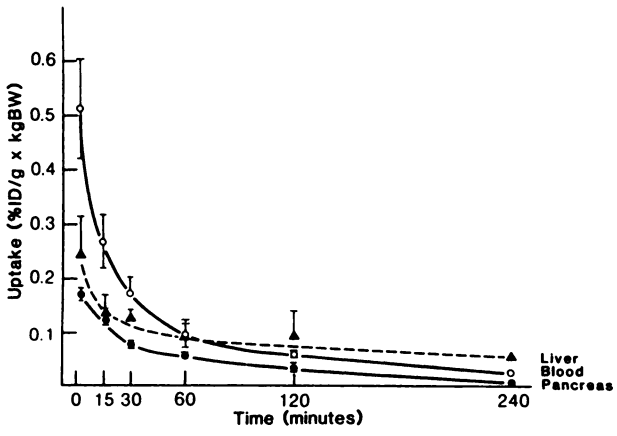

FIGURE 4
The uptake of ^{153}Gd (injected as [^{153}Gd]DTPA) is shown as a function of time for liver, blood, and pancreas. Results are expressed as the mean ± s.e.m. of three dogs.

The relatively low uptake of [^{54}Mn]DTPA in canine myocardium is of particular interest since the acute toxicity of manganese is primarily related to the cardiovascular system. Our value of 0.2% of the injected [^{54}Mn]DTPA retained in the myocardium at 4 hr is 1/10 the previously published value for [^{54}Mn]$MnCl_2$ (*12*). The myocardium-to-blood ratios were 7.3:1 for [^{54}Mn]DTPA while they were 70.5:1 for [^{54}Mn]$MnCl_2$. The absolute liver uptake of [^{54}Mn]DTPA was 12.6% of the injected dose compared with 41.4% reported for [^{54}Mn]$MnCl_2$ (*12*). Comparing the heart-to-liver ratios they were 0.26:1 for [^{54}Mn]$MnCl_2$ while they were 0.047:1 in our experiments with [^{54}Mn]DTPA.

The ultimate clinical utility of complexes such as manganese DTPA for NMR contrast enhancement remains to be determined. Detailed acute and chronic toxicological investigations will have to be conducted before use in humans could be contemplated. However, since our results have shown that [Mn]DTPA accumulates in the liver, pancreas, bowel, and kidneys, and also alters the NMR signal strength of the hepatobiliary and renal systems in dogs, we believe that these toxicity experiments are worth conducting.

NOTES

* Sigma Chemical Company, St. Louis, MO.
† DuPont Company, Boston, MA.

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REFERENCES

1. Brasch RC. Work in progress: methods of contrast enhancement for NMR imaging and potential applications. *Radiology* 1983; 147:781–788.
2. Weinmann HJ, Brasch RC, Press W, et al. Characteristics of gadolinium-DTPA complex: a potential NMR contrast agent. *Am J Roentgenol* 1984; 142:619–624.
3. Goldstein EJ, Burnett KR, Hansell JR, et al. Gadolinium DTPA (an NMR proton imaging contrast agent): chemical structure, paramagnetic properties and pharmacokinetics. *Physiol Chem Phys* 1984; 16:97–104.
4. Gries H, Miklautz H. Some physiochemical properties of the gadolinium-DTPA complex, a contrast agent for MRI. *Physiol Chem Phys* 1984; 16:105–112.
5. Wolf GR, Burnett KR, Goldstein EJ, et al. Contrast agent for magnetic resonance imaging. *Mag Res Ann* 1985; 231–266.
6. Runge VM, Clanton JA, Price AC, et al. Paramagnetic

contrast agents in magnetic resonance imaging: research at Vanderbilt University. *Physiol Chem Phys* 1984; 16:113–122.
7. Wesbey GE, Engelstad BL, Brasch RC. Paramagnetic pharmaceuticals for magnetic resonance imaging. *Physiol Chem Phys* 1984; 16:145–155.
8. Laniado M, Weinmann HJ, Schorner W, et al. First use of Gd DTPA/dimeglumine in man. *Physiol Chem Phys* 1984; 16:157–165.
9. Boudreau RJ, Frick MP, Levey RM, et al. The preliminary evaluation of Mn-DTPA as a potential contrast agent for nuclear magnetic resonance imaging. *Am J Physiol Imaging* 1986; 1:19–25.
10. Kent NL, McCance RA. The absorption and excretion of "minor" elements by man. *Biochem J* 1941; 35:877–883.
11. Hauser W, Atkins HL, Nelson KG, et al. Technetium-99m DTPA: a new radiopharmaceutical for brain and kidney scanning. *Radiology* 1970; 94:679–684.
12. Chauncey DM, Heinrich RS, Halpern SM, et al. Tissue distribution studies with radioactive manganese: a potential agent for myocardial imaging. *J Nucl Med* 1977; 18:933–936.
13. Utter MF. The biochemistry of manganese. *Med Clin NA* 1976; 60:713–727.
14. Borg DC, Cotzias GC. Manganese metabolism in man: rapid exchange of Mn-56 with tissue as demonstrated by blood clearance and liver uptake. *J Clin Invest* 1958; 37:1269–1278.
15. Maynard LS, Cotzias GC. The partition of manganese among organs and intracellular organelles of the rat. *J Biol Chem* 1954; 214:489–495.