PRELIMINARY NOTE

Use of a Paramagnetic Substance, Colloidal Manganese Sulfide, as an NMR Contrast Material in Rats

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Paramagnetic pharmaceuticals (magnetopharmaceuticals) that are suitably distributed into specific organ systems or diseased sites might be clinically useful for tissue contrast enhancement in nuclear magnetic resonance images. To determine whether an insoluble magnetopharmaceutical might be useful in such service, we investigated the effect of a colloidal preparation of manganese sulfide (MnSC) upon liver and lung spin-lattice relaxation times (T_1) in rats following intravenous administration. NMR tissue sample measurements were made at 24 MHz, and showed that after MnSC treatment, liver T_1 values—and to a lesser extent lung T_1 values—were depressed below control values. Liver manganese content (as determined by flame atomic absorption spectrophotometry) increased in proportion to the dose of MnSC, and the reciprocal of the liver T_1 values also increased in proportion to the dose of MnSC.

J Nucl Med 25: 604–607, 1984

Using nuclear magnetic resonance (NMR), one can obtain high-resolution diagnostic images of human anatomy and disease without the use of ionizing radiation. The ability of NMR to provide these images is due to the differences in tissue relaxation times that normally exist among healthy tissues of most organ systems. Additionally, pathologic changes in tissues, such as edema, inflammation, and tumor, produce characteristic changes in tissue water content; these alter tissue relaxation times and permit their detection by NMR.

To date, the diagnostic information is usually of an anatomic nature, but there is the likelihood that physiologic conditions can be portrayed by NMR imaging, particularly if suitable paramagnetic pharmaceuticals (magnetopharmaceuticals) can be developed to behave as physiologic substrates. Magnetopharmaceuticals could also prove valuable in anatomic visualization of organs or tissues that have only small differences in relaxation times compared with surrounding tissues and cannot be clearly visualized by NMR. Researchers have begun to investigate a paramagnetic element, manganese, for tissue contrast enhancement in NMR (*1*). Currently, however, there are no reports on the usefulness of colloidal (insoluble) species of paramagnetic elements as NMR contrast agents. In nuclear medicine, Tc-99m sulfur colloid, a radiopharmaceutical that is taken up specifically by cells of the reticuloendothelial (RE) system, is routinely used for liver, spleen, and bone marrow imaging (*2*). By analogy with Tc-99m sulfur colloid, we guessed that a colloidal preparation of a paramagnetic element such as manganese might be useful as an NMR contrast agent for studies of the RE system. In this paper we report the effects of intravenously administered colloidal manganese sulfide (MnSC) upon the NMR spin-lattice relaxation time and manganese content of rat liver.

Received July 13, 1983; revision accepted Nov. 21, 1983.

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MATERIALS AND METHODS

Paramagnetic tracer. Colloidal manganese sulfide was prepared by the coprecipitation of equal molar quantities of manganese acetate and sodium sulfide. The preparation was characterized as a salmon-colored suspension that was observed in scanning electron micrographs to have a particle size of 0.1–10 μ. The amount of noncolloidal manganese in the preparation was determined by filtration using a 0.22-μ Millipore filter, then comparing

TABLE 1. EFFECT OF INTRAVENOUSLY ADMINISTERED COLLOIDAL MANGANESE SULFIDE UPON RAT LIVER T_1 VALUES [EXPRESSED AS $1/T_1$ (sec^{-1})] AND LIVER Mn CONTENT. RATS WERE KILLED 30 min AFTER I.v. DOSE

Average dose MnSC (mg/kg)	N	Mean $1/T_1$ (sec^{-1}) (range)	Mean liver Mn content (mg/kg)* (range)
Controls	3	3.26 (3.17–3.35)	4.4 (3.6–5.9)
0.37	2	4.10 (4.03–4.15)	6.6 (6.3–6.8)
0.68	2	5.20 (4.76–5.62)	18.0 (17–19)
0.83	3	5.13 (4.18–5.95)	22.0 (15–28)
0.91	5	5.30 (4.5–6.43)	20.5 (13–29)
1.08	2	6.50 (5.06–7.94)	NA
1.48	3	8.46 (7.2–9.8)	36.7 (35–38)
1.97	3	7.82 (7.58–8.06)	40.1 (32–50)
3.00	4	12.40 (10.56–13.3)	58.5 (54–65)

* Liver dry weight.

the T_1 signal of the filtrate against those of known dilutions of manganese acetate. In this way it was determined that at least 95% of the preparation had a particle size greater than 0.22 μ, but since smaller colloidal particles will pass this filter, it is likely that the percentage of soluble manganese in the preparation was actually less than 5%.

Animals. Male Sprague-Dawley rats weighing approximately ~225 g were used.

NMR techniques. Relaxation times were determined using a pulse spectrometer* operating at 24 MHz. This device uses a single solenoidal transmitter/receiver coil that is 14 mm long by 7.5 mm diam. The instrument uses a crystal-controlled oscillator, which prevents any variation of the frequency of the spectrometer. The magnet† had a field stability of 3×10^{-6}. The spectrometer's probe head was kept at 22°C. T_1 was measured using a $180° - \tau - 90°$ pulse sequence. The initial height of the free induction decay after the 90° pulse was taken to be the longitudinal component of magnetization, $M(\tau)$. The signal-to-noise ratio for the spectrometer was sufficiently high so that signal averaging was not necessary to obtain $M(\tau)$. A least squares fit was made to the function $\ln [(M_0 - M(\tau)/2M_0)]$ compared with τ, with M_0 being the equilibrium value, i.e., for $\tau > 5\ T_1$. Measurements of the magnetization were obtained for at least seven values of τ in each case. The correlation coefficient for the least squares fit was never less than 0.99. The reciprocal of the slope of the least squares fit line was taken to be T_1.

Although this work is primarily concerned with the effect upon T_1, a number of measurements of T_2 were obtained from treated and control rat tissues. T_2 values were obtained by observing the decay of a Carr-Purcell spin-echo train (*3*) with the Meiboom-Gill modification (*4*). In this spin-echo train, the spacing between 180° pulses was 5 msec. A least squares fit was made to the logarithm of the echo amplitude compared with the time of appearance of the echo after the initial 90° pulse. The reciprocal of the slope of this line was taken to be T_2.

Experimental protocol. Rats were intravenously injected with MnSC in dosage levels of 0.37–3.0 mg manganese sulfide per kg body weight. The animals were killed ~30 min after injection, tissue samples (liver, lung, and blood) were removed, and T_1 measurements were obtained.

Manganese content in liver and lung tissues was determined on lyophilized tissues using a flame atomic absorption spectrophotometer. A hollow-cathode lamp was used for manganese detection in absorbance mode at 279.5 nm. The dry weight of liver samples was determined and samples were extracted with 1% nitric acid by a procedure similar to that used by Hinners (*5*). Manganese content of liver tissues from both treated animals and controls was determined by a comparison of sample analysis with a calibrated curve from manganese standards.

RESULTS

Table 1 details the effect of specific dosages upon liver T_1 values in rats (expressed as the reciprocal value) and the liver manganese content, as analyzed from control and MnSC treated animals. According to theory, the reciprocal of T_1 should be proportional to the concentration of paramagnetic ions (*6*). Compared with liver T_1 values in control animals (307 msec), MnSC caused a decrease of liver T_1 measurements, which then ranged from 244 msec (0.4 mg MnSC/kg) to 81 msec (3 mg MnSC/kg). The reciprocal of T_1 correlated highly with increasing doses of MnSC (Fig. 1). Liver manganese content, as determined by flame atomic absorption data,

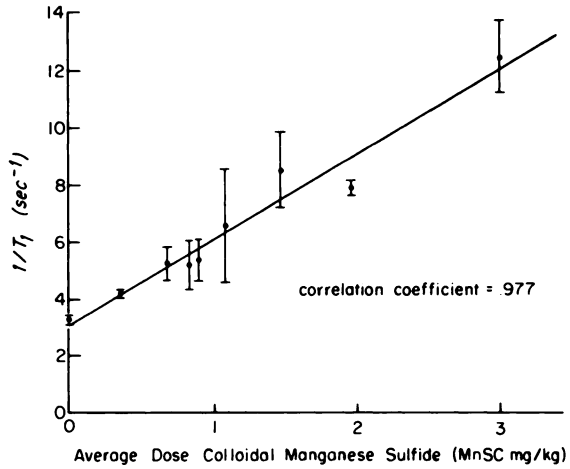

FIG. 1. Effect of colloidal manganese sulfide (MnSC) upon T_1 measurements [plotted as $1/T_1$ (sec^{-1})]. Bars show approximate ranges.

TABLE 2. EFFECT OF COLLOIDAL MANGANESE SULFIDE UPON T_1 MEASUREMENTS OF BLOOD, LIVER, AND LUNG TISSUES WHEN RATS WERE KILLED AT 30 AND 60 min AFTER DOSE (0.8 mg MnSC/kg), ALONG WITH CONTROL T_1 VALUES. VALUES EXPRESSED AS $1/T_1$ sec^{-1}, WITH RANGE IN PARENTHESES

Treatment: Kill Time	Mean $1/T_1$ (sec^{-1}) (range) Blood	Liver	Lung
30 min	1.05	4.79	2.65
N = 2	(1.057–1.061)	(4.6–4.98)	(2.59–2.72)
60 min	1.065	4.22	1.93
N = 3	(1.02–1.11)	(4.11–4.32)	(1.85–1.98)
Controls	—	3.63	1.49
N = 3		(3.4–3.8)	(1.46–1.51)

also correlated well (r = 0.988) with the amount of MnSC administered (Fig. 2). Liver manganese content and the reciprocal of T_1 for liver tissues were also highly correlated (r = 0.977).

The manganese content of livers from control animals was about 4.4 mg manganese/kg dry weight and was below those levels established by the National Bureau of Standards (NBS) for dessicated rat livers. The percentage of intravenously administered MnSC removed by rat liver was determined to be 40–50%.

The T_1 values for lung tissues from both treated and control animals are shown in Table 2. Compared with controls, there were lower T_1 values in lung and liver tissues from treated animals, but when the kill time was increased from 30 min to 60 min after MnSC administration, the T_1 values for both these tissues returned closer to control values. The control values we obtained were in excellent agreement with the values for rabbits reported by Ling (*7*).

A limited number of T_2 measurements were made. In rat liver we observed a decrease in T_2 that was similar to the T_1 decrease, only much smaller in degree. For example, the MnSC dose that decreased liver T_1 from 300 msec to 100 msec ($1/3$ of control values) correlated with a T_2 decrease from 40 msec to 28 msec (a decrease to only $2/3$ of the control values).

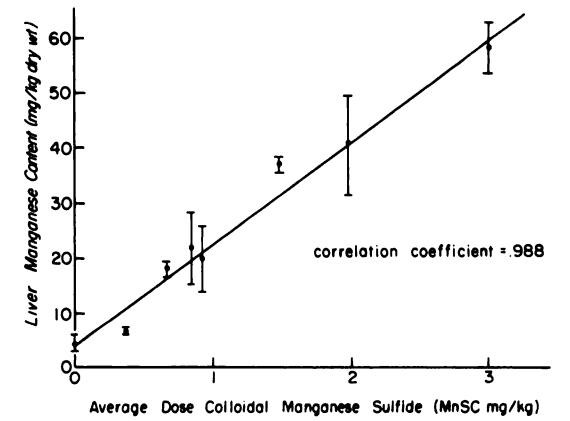

FIG. 2. Liver manganese content as determined by flame atomic absorption spectroscopy versus dosage of colloidal manganese sulfide (MnSC). Bars show approximate ranges.

DISCUSSION

Several researchers have investigated the effects of paramagnetic substances upon relaxation times (*8,9*). To date, complexes of paramagnetic metal ions appear to offer the most promise (*10,11*) for the development of magnetopharmaceuticals. Manganese is one of the more favorable of these elements because it very effectively alters relaxation times (*12*), its concentration in tissues can be accurately quantified by flame atomic absorption spectroscopy (*5*), and it is only moderately toxic (*13*). In studies by Wolf and Baum (*14*) and Lauterbur et al. (*1*), manganese ions (administered as soluble salts) significantly alter T_1 in organs of animals, most notably the liver, heart, and kidneys. The effects they observed upon T_1 appear dose-related, since tissues with high concentrations of manganese have proportionately greater reduction in T_1 measurements. One group suggests that the manganese T_1 dose response, therefore, is really a measure of manganese biodistribution at the time of animal sacrifice (*14*).

It is likely that one could make a colloidal preparation of manganese that has biodistribution characteristics similar to that of technetium-99m sulfur colloid, an agent used in nuclear medicine for imaging the liver, spleen, and bone marrow. In this study, rats receiving colloidal manganese sulfide had markedly lower T_1 values than controls, and the reciprocal of these T_1 values very closely correlated to the amount of drug administered and the amount of manganese in the tissues. The precise mechanism by which this insoluble manganese species causes a T_1 shift is not known. Since Tc-99m sulfur colloid is

phagocytized by Kupffer cells and remains fixed there indefinitely, it is suspected that the analogous manganese colloid may be similarly phagocytized by these cells, and that any alteration upon T_1 may occur from some type of intracellular effect by MnSC.

The observed effect of MnSC upon lung T_1 may result from localization through pulmonary capillary blockade by MnSC particles that are larger than red blood cells (7–8 μ). Scanning electron micrographs of the preparation had previously demonstrated MnSC particles at least as large as 10 μ in diameter.

In conclusion, colloidal manganese sulfide can be used to alter T_1 values in rat liver, thus producing contrast enhancement in these tissues. The term "contrast agent" is not new in medicine, since contrast materials have been used in radiology for several years to enhance the observations of organs or tissues that would otherwise not appear in routine x-ray films, or be poorly visualized at best. Contrast enhancement in NMR imaging may further NMR's potential advantage over other diagnostic modalities. Whether colloidal manganese sulfide will prove clinically useful is not known, since studies on manganese toxicity have not evaluated the long-term effect of colloidal species. Regardless, this study demonstrates that colloidal manganese sulfide can affect T_1 measurements in rat liver. Further studies are needed to determine the applicability of this agent for clinical use.

FOOTNOTES

* Spin-Locke Ltd., Mississauga, Ontario, Canada.

† Walker Scientific Inc., Worcester, Massachusetts.

ACKNOWLEDGMENTS

The authors thank Mr. David Thompson and Mrs. Christine Johnston for their valuable technical assistance, and Ms. Nina Haynes and Ms. Jan Rogers for preparing the manuscript.

This study was supported by a research grant from the Department of Radiology.

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