Investigative Radiology

T1 relaxivities of Gadolinium-Based Magnetic Resonance Contrast Agents in Human Whole Blood at 1.5, 3 and 7 Tesla

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Abstract:	Objectives Calculation of accurate T1 relaxivity (r1) values for gadolinium based MR contrast agents (GBCAs) is a complex process. As such, often referenced r1 values for the GBCAs at 1.5T, 3T, and 7T are based on measurements obtained in media that are not clinically relevant, derived from only a small number of concentrations, or available for only a limited number of GBCAs. This study derives the r1 values of the eight commercially available GBCAs in human whole blood at 1.5T, 3T and 7T. Materials and Methods Eight GBCAs were serially diluted in human whole blood, at 7 concentrations from 0.0625 to 4mmol/L. A custom-built phantom held the dilutions in air tight cylindrical tubes maintained at 37±0.5℃ by a heat circulating system. Images were acquired utilizing inversion recovery sequences with TIs from 30ms to 10s at 1.5, 3T, and 60ms to 0.5s at 7T. A custom Matlab program was used to automate signal intensity measurements from images acquired of the phantom. SigmaPlot was used to calculate T1 relaxation times and finally r1. Results Measured r1 values in units of L•mmol-1s-1 at 1.5T(3T/7T) were 3.9±0.2 (3.4±0.4/2.8±0.4) for Gd-DOTA, 4.6±0.2 (4.5±0.3/4.2±0.3) for Gd-DO3A-butrol, 4.3±0.4 (3.8±0.2/3.1±0.4) for Gd-DTPA, 6.2±0.5 (5.4±0.3/4.7±0.1) for Gd-BOPTA, 4.5±0.1 (3.9±0.2/3.7±0.2) for Gd-DTPA-BMA, 4.4±0.2 (4.2±0.2/4.3±0.2) for Gd-DTPA-BMEA, 7.2±0.2 (5.5±0.3/4.9±0.1) for Gd-EOB-DTPA, and 4.4±0.6 (3.5±0.6/3.4±0.1) for Gd-HP-DO3A. The agents can be stratified by relaxivity, with a significant additional dependency on field strength. Conclusions This report quantifies for the first time T1 relaxivity for all eight gadolinium chelates in common clinical use worldwide, at current relevant field strengths, in human whole blood at physiological temperature (37℃). The measured r1 values differ to a small degree from previously published values, where such comparisons exist, with the current r1 measurements being that most relevant to clinical practice. The macrocyclic agents, with the exception of Gd-DO3A-butrol, have slightly lower r1 values when compared to the two much less stable linear agents, Gd-DTPA-BMA and Gd-DTPA-BMEA. The two agents with hepatobiliary excretion, Gd-EOB-DTPA and Gd-BOPTA,

	have at 1.5 and 3T substantially higher r1 values than all other agents.

Dear Editors:

We would like to submit the enclosed manuscript entitled “T1 relaxivities of Gadolinium-Based Magnetic Resonance Contrast Agents in Human Whole Blood at 1.5, 3 and 7 Tesla ”, which we wish to be considered for publication in Investigative Radiology. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

In this work, we evaluated the T_1 relaxivity (r_1) of eight commercially available GBCAs in human whole blood at 1.5T, 3T and 7T. Calculation of accurate T_1 relaxivity (r_1) values for gadolinium based MR contrast agents (GBCAs) is a complex process. As such, often referenced r_1 values for the GBCAs at 1.5T, 3T, and 7T are based on measurements obtained in media that are not clinically relevant, derived from only a small number of concentrations, or available for only a limited number of GBCAs. This study derives the r_1 values of eight commercially available GBCAs in human whole blood at 1.5T, 3T and 7T. I hope this paper is suitable for Investigative Radiology.

We deeply appreciate your consideration of our manuscript, and we look forward to receiving comments from the reviewers. If you have any queries, please don’t hesitate to contact me at the address below.

Thank you and best regards.

Yours sincerely,

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Dear Dr. Tweedle,

Thank you very much for your letter and comments from the reviewers for their careful reading, comments and suggestions.
We have checked the manuscript and revised it according to the comments. For the individual comments see our reply below.
If you have any question about this paper, please don't hesitate to let me know.

Sincerely yours,

Reviewer #1:
The paper provides a valuable reference of the T1-relaxivities of all but one commercial GBCA in human blood. This is certainly the most relevant medium in which these measurements can be performed, although it is technically demanding to perform these measurements in correct way.
A few aspects of the work require a more detailed description to really understand what was done and how the results were obtained. This is especially necessary because the authors used several protocols and methods for evaluation and it is not always clear which combination of protocols and methods were used to obtain the final results and why they discarded the other results.
Abstract:
"?TIs from 30ms to 10s at 1.5, 3, and 7T?.". This is not correct. At 7T the shortest TI was 60 ms.
Thank you for noticing this, we have corrected it.
"?Measured r1 values were compared to those in previous publications with paired t-tests." Such comparison by T-test is not reported in the result section.
Yes we mistakenly put this in the abstract and it has been removed
Introduction:
Magnevist was first approved in 1988 not 1986.
This has been corrected
The authors excluded MS325 form their measurements. They should give a reason for this. It is really a pity that they excluded it, first because this leaves their work incomplete and second because the concentration dependence of the relaxivity of MS325 is very different to all other agents.
We decided to exclude MS-325 because it is no longer on the market in Europe and is not used widely in other areas of the world. We have added this in the introduction.
Methods and Material:
The authors mention the difference in the blood batches which they used and that they accounted for them. But it remains unclear how they did this. Was the blood diluted to obtain the same hematocrit? Or were the differences too small to care about. The authors should explain this in more detail.
There was a difference in Hct between the two sources of blood. After measuring both sources

with the same protocol at 3T we found no significant differences in r_1, indicating that Hct concentration has little to no effect on our measurements. This is now explained more thoroughly in the manuscript.

Imaging protocols:
At 1.5 an 3 T the authors used 2 different protocols with different TI times for samples with short and long T1-times. It is not clear from the description whether all samples were measured with all protocols or whether the samples were split in 2 groups and were measured only with one of them, according to the expected T1-time.
All samples were measured with all protocols. This has been added to the article. We originally anticipated the need for 2 protocols because our sample had short and long T1-times. It turned out that both protocols gave similar results at each Gd concentration but we reported them regardless.

Two important limitations need to be mentioned.
1. The shortest TI was 30 ms. For the 4 mM samples T1-times in the range of 40-50 ms are reached. To measure these times with the same accuracy as the samples with longer T1-times TI must be much shorter than 30 ms. Otherwise there are only 2-3 data point on the curved part of the relaxation curve. Therefore the authors should also provide a figure like 2B for the 4 mM sample of Gd-EOB-DTPA at 1.5 and 3T. This is even worse at 7T where 60 ms was the shortest TI.
Revised figures were provided in Figure 2B and 2C.
2. In the Discussion the authors mention correctly that TR must be at least 3 x T1. However, they set TR to 1.5 and 4 sec. At 1.5T Gd-DOTA provides a T1 time at 0.0625 mM of about 1 sec and at 3T of even 1.2 sec. At least for these samples and also for the blank blood with T1-times of 1.3 to 1.7 sec the used TR are too short.
At 7T the selected TR=3s is also too short for the blank blood and samples with low conc.
Actually, protocol 2 is doubling the scanning time of protocol 1. We meant to find out whether there is difference between the final results. Table 2 and 3 prove that, there isn't a significant difference exit. We found through trial and error that long TRs made it impossible to maintain the temperature and prevent the solution from settling before the scans were complete.

The authors should explain how they converted the measured signal intensities in negative numbers. Usually SI is always a positive number and the resulting curve looks very different to the one presented in Fig 2B and 3. See the following figure as an example
This is now explained. "Since the signal intensities were all positive because the scanner outputs magnitude data the inflection point was chosen so the curve best fit equation (1). Thus positive numbers below the inflection point along the x-axis were converted to negative."

(send by email to the editor!)

The authors mention a curve fit for the determination of r1. They should specify what curve fit was used. If it was a simple unweighted linear regression the problem is that the highest concentration with the highest R1 dominates the slope of the curve and thus r1. This is clearly seen on all Fig. 5 plots. This is very problematic, because, as mentioned above, the R1 of the highest concentrations were determined with the least accuracy, because of the TI limitation. It would be more appropriate to either use a 1/Y weighted linear regression or calculate r1 individually for each concentration and then calculate the mean ± SD.
Calculating r1 for each concentration will add more error to the results this is because r1 error will be heavily attributed to the concentration of Gd in our samples. This error will be propagated to each sample because they are serial dilutions and further increase error. We calculated r1 this way as we found it would produce the results with the least amount of error.

The authors should explain more clearly the rationale to use three different methods to calculate r1. It remains unclear which samples (concentrations and from which protocol) were included in which method and which method is finally the most appropriate one.
A more clear explanation has been added in the materials and methods section.

Statistical Analysis: The authors compare Protocol 1 and 2 for a specific set of samples. Why was this done and which of the methods I to III were used.
This is now explained

The different routes to calculate and compare r1 are very confusing and should be explained more clearly or the authors should restrict themselves to one method that produced the most reliable results.
We further clarify this now

Results:
The authors find some remarkable differences in the R1 of blank blood, especially at 3T. But they do not draw any conclusions from this finding. Does it have any relevance or influence on the presented results? This is also not addressed in the discussion.
We now address this in the discussion.

Presentation of r1 in Table 5. Which one of the three values for 1.5 and 3 T is the final relevant value? Probably the value that was included in table 6. But why was it this value and not one of the others?
This is now explained in the results and discussion. We found results from method II to be most accurate.

Discussion:
p14: "?To account for this, three different methods were used to calculate r1. All the results were similar (Table 5). For all eight GBCAs, results calculated with method ?, which ?" The authors should present a conclusion, which of the 3 methods is most appropriate. What is the teaching that method II is not different to the other methods, although it is restricted to only 3 of the 7 concentrations.

Method II used all 7 concentrations We now explain this choice in the results and in the discussion

Figure 2C: which compound, which field strength?
Figure 2B: which protocol?
Figure 2 to 4. Inversion time should be in the same unit. The unit should be given in brackets.
Figure 4B and C: There are 2 or 3 data sets plotted on top of each other. This needs to be explained in the legend.
Figure 5: unit of concentration should be given in brackets.
These changes have now been made.

Reviewer #2:
This is a useful study of most of the available GBCA at three field strengths, reporting r1 in human blood.
Abstract: There is nothing in this report re: the stability of GBCA and the abstract has no real business making this differentiation, even though it is factual.
We have now changed this statement
1)Magnevist was approved in its first country in 1988, not 1986.
This change has been made thank you.

2)why exclude Ablavar. It is available, although very seldom used. It is particularly interesting as kits relaxivity is known to be more nonlinear with concentration than other agents.
We now explain this in the introduction.

3)technically, 1/T1 is a relaxation rate "constant" so defining relaxivity as the change in rate with concentration is not rigorously correct. "Change in reciprocal relaxation time per unit concentration" is rigorously correct, and "Change in relaxation rate as measured by reciprocal relaxation time per unit concenbtration" is correct. (Rate is dx/dt and has units of velocity, while T1, T2 have units of time.)
We have now changed this to reflect your suggestion. Thank you, we agree this is more precise terminology.

4)p3 again, another problem with earlier studies was failure to account for nonlinear 1/T1,2 vs [Gd] when proteins are present in the protein binding agents. When this is the case relaxivity is no longer measurable by the slope of reciprocal T1,2 vs [Gd].
We agree that relaxivity is not entirely linearly especially in the case of protein binding. This study did not seek to determine linearity but only provide the most accurate measures of relaxivity based on previous conclusions that relaxivity is a linear phenomenon.

5) Optional correction: On p 20, "The longitudinal relaxation rate R1 was calculated" does not define R1. It is simply the reciprocal of T1, and technically is not the "rate." It can

"represent the rate," if authors want to be accurate, but do not want to stray from the incorrect convention that most researchers use (i.e. calling R1 a "rate" instead of the more correct, "rate constant.") Another way to handle it is to use (technically R1 is a rate constant, but we will abbreviate it), or an endnote.
We made these changes as you suggested, again we belive you are correct and this is more precise, thank you.

6)p3 last para. Excluding ms325 because it is "protein bound" ignores the fact that gadobenate and Eovist also bind proteins. Both of these agents have fairly regular usage, however, while ms325 has almost no usage.

Thank you for noticing these(1-6), we have corrected it and added into our paper.

6)How consistent and accurate are the manufacturers' concentrations? This would affect measured values. If the samples of the original bottles are available, or even the lot numbers, ICP or the manufacturers could determine the stock solution concentrations. Manufacturers probably measure the [Gd] on product batches.
ICP results of the original bottles have now been added to the paper.

7) "Relaxivity is defined as the rate at which R1 changes with a change in GBCA concentration, in other words, the slope of the line obtained by plotting R1 against the concentrations of contrast agent(1)." This is what happens when you start with an incorrect definition of "rate." r1 is a rate constant, not a rate. To be a rate it would need velocity units, and mM-1s-1 are not velocity units. These are the units of a second order rate constant, just as s-1 are the units of a first order rate constant (R1). Plotting the first order rate constant against concentration yields a line (absent protein binding), the slope of which is the second order rate constant, r1, the relaxivity. (In the presence of protein binding one cannot r1 by a slope, but it is defined by calculating it at the individual [Gd] and must be presented with that [Gd]. (ref. de Haën, Christoph (05/2002). "The problematic determination of proton magnetic relaxation rates of protein-containing solutions". Academic radiology (1076-6332), 9 Suppl 1 (1), p. S2.) The cleanest way to state these definitions is to stick to the definition that "r1 is the second order rate constant defining the ability of any GBCA to catalyze water proton relaxation." See ref. 1 of this paper.

We have made the suggested change. We now refer to R_1 as relaxation rate constant and state that "Relaxivity is the second order rate constant defining the ability of any GBCA to catalyze water proton relaxation"

8)Tables, the abbreviation for seconds is a lower case s, not an upper case S. Also figures.
This has now been changed

9)Table 5. Comparison of r1 (L.S-1.mmol-1) abbreviation s, not S. It would be more conventional to report units as s-1mM-1 where M is the internationally recognized SI unit abbreviation for Molar, that is moles/L, hence mM is millimolar. Unfortunately, radiologists

sometimes mistakenly use M for mole instead of mole/L when they quote Gd doses for in vivo use.
This has now been changed
10)Figures. Fig 4 C use the wrong abbreviation for seconds. Use s. Fig 5 lacks the units on the text for the X axis. This reviewer cannot see Figure 6.
From8-10,these changes have now been made.

11) Pintaske published an extensive Erratum that authors seem to have missed or at least not referenced. It is in Invest. Radiol. And the table herein is from the erratum. Pentaske's data are nonlinear at a concentration well above the linear range experienced herein and in Pentaske at lower concentrations. The r1 published below are closer to authors' r1 and are the official Pentaske data now that he has corrected his earlier paper with an erratum.
Investigative Radiology Issue: Volume 41(12), December 2006, p 859
Thank you for pointing this out, and we are adding this in our paper and have updated our tables

12)another reason for discrepancies could be the concentration ranges used in different studies, even in the same media. If the data are truly nonlinear, lower concentration data will generally give higher r1 values. Also 1.5T data will give higher values, both of these because nonlinearity is presumed to be due to slight to moderate protein binding. Immediate injection of 7 mmol of Gadobenate into 3 L of plasma in vivo would produce 2 mM, so that at physiologic use ranges, linearity is not a poor approximation of the truth. Rat studies also are only comparable if the binding of a protein binding GBCA to rat albumin is the of the same magnitude as binding to human albumin. Albumin binders can be selective for species.
Thank you for pointing this out, and we are adding this in our paper.

T_1 relaxivities of Gadolinium-Based Magnetic Resonance Contrast Agents in Human Whole Blood at 1.5, 3 and 7 Tesla

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T_1 relaxivities of Gadolinium-Based Magnetic Resonance Contrast Agents in Human Whole Blood at 1.5, 3 and 7 Tesla

Abstract

Objectives Calculation of accurate T_1 relaxivity (r_1) values for gadolinium based MR contrast agents (GBCAs) is a complex process. As such, often referenced r_1 values for the GBCAs at 1.5T, 3T, and 7T are based on measurements obtained in media that are not clinically relevant, derived from only a small number of concentrations, or available for only a limited number of GBCAs. This study derives the r_1 values of the eight commercially available GBCAs in human whole blood at 1.5T, 3T and 7T.

Materials and Methods Eight GBCAs were serially diluted in human whole blood, at 7 concentrations from 0.0625 to 4mmol/L. A custom-built phantom held the dilutions in air tight cylindrical tubes maintained at 37±0.5℃ by a heat circulating system. Images were acquired utilizing inversion recovery sequences with TIs from 30ms to 10s at 1.5, 3T, and 60ms to 0.5s at 7T. A custom Matlab program was used to automate signal intensity measurements from images acquired of the phantom. SigmaPlot was used to calculate T_1 relaxation times and finally r_1. ~~Measured r_1 values were compared to those in previous publications with paired t-tests.~~

Results Measured r_1 values in units of $L \cdot mmol^{-1}s^{-1}$ at 1.5T(3T/7T) were 3.9±0.2 (3.4±0.4/2.8±0.4) for Gd-DOTA, 4.6±0.2 (4.5±0.3/4.2±0.3) for Gd-DO3A-butrol, 4.3±0.4 (3.8±0.2/3.1±0.4) for Gd-DTPA, 6.2±0.5 (5.4±0.3/4.7±0.1) for Gd-BOPTA, 4.5±0.1 (3.9±0.2/3.7±0.2) for Gd-DTPA-BMA, 4.4±0.2 (4.2±0.2/4.3±0.2) for Gd-DTPA-BMEA, 7.2±0.2 (5.5±0.3/4.9±0.1) for Gd-EOB-DTPA, and 4.4±0.6 (3.5±0.6/3.4±0.1) for Gd-HP-DO3A. The

agents can be stratified by relaxivity, with a significant additional dependency on field strength.

Conclusions This report quantifies for the first time T1 relaxivity for all eight gadolinium chelates in common clinical use worldwide, at current relevant field strengths, in human whole blood at physiological temperature (37℃). The measured r_1 values differ to a small degree from previously published values, where such comparisons exist, with the current r_1 measurements being that most relevant to clinical practice. The macrocyclic agents, with the exception of Gd-DO3A-butrol, have slightly lower r_1 values when compared to the two much less stable linear agents, Gd-DTPA-BMA and Gd-DTPA-BMEA. The two agents with hepatobiliary excretion, Gd-EOB-DTPA and Gd-BOPTA, have at 1.5 and 3T substantially higher r_1 values than all other agents.

Key words: relaxivity; MRI contrast media; gadolinium; whole blood; field strength dependence; 1.5T; 3T; 7T comparative studies

Introduction

Gadopentetate dimeglumine (Magnevist, Gd-DTPA) was the first gadolinium-based MR contrast agent (GBCA) approved for clinical use, which occurred in ~~1986~~1988. To date, nine GBCAs have been approved for use in Europe and the United States.[1, 2]~~(1, 2)~~ The additional eight include Gd-BOPTA (gadobenate dimeglumine, MultiHance), Gd-DO3A-Butrol (gadobutrol, Gadovist/Gadavist), Gd-DOTA (gadoterate meglumine, Dotarem), Gd-DTPA-BMA (gadodiamide, Omniscan), Gd-DTPA-BMEA (gadoversetamide, OptiMARK), Gd-EOB-DTPA (gadoxetic acid disodium, Primovist/Eovist), Gd-HP-DO3A (gadoteridol, ProHance), and MS-325 (gadofosveset trisodium, Ablavar). In clinical practice, the GBCAs are most commonly utilized to improve detection and differentiation of pathological lesions and to visualize the vasculature in magnetic resonance angiography applications.

Relaxivity is a critical parameter in determining the relative efficacy of the GBCAs. Relaxivity is defined as the change in reciprocal relaxation ~~rate~~ time per unit concentration of Gd chelate. T_1 relaxivity, known as r_1, is influenced by many variables including magnetic field strength, temperature, environmental conditions, and protein concentration.[1, 3-5]~~(1, 3, 4)(5)~~

Previous in vitro studies have examined the relative relaxivities of the gadolinium chelates. However, these have been incomplete—not including all relevant field strengths or deriving calculations from a wide range of concentrations—or inapplicable to human imaging—not performed in human whole blood under at physiologic temperatures, or failing to account for nonlinear 1/T1 vs [Gd] when proteins are present in the protein binding agents..[3, 4, 6-10]~~(3, 4, 6-10)~~

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This study aims to address these limitations by determining the T_1 relaxivity values across a wide range of concentrations at 1.5T, 3T, and 7T for the eight primary, commercially available, GBCAs (excluding MS-325, which is strongly protein bound, seldom used in clinical work in most of the world and no longer commercially available in Europe) in human whole blood at 37℃.

Materials and Methods

Preparation of human whole blood samples

Whole human blood was acquired from a commercial supplier (Valley Biomedical Products & Services, Inc.). Due to 7 Tesla scanners not being widely available, the experiments were performed at two different sites—one housing the 1.5 and 3 Tesla scanners (site 1) and the other housing the 7 Tesla scanner (site 2). This also required a different human blood sample to be utilized at each respective site because it was not possible to preserve the first sample set for use at the second site. For standardization purposes, hematocrit fractions (Hct) of each sample set were ~~thus~~ measured prior to dilution (site 1: Hct was 50 ± 0.5, site 2: Hct was 45 ± 0.5). To ensure ~~uniformity~~ relaxivity results were not affected by the different Hct concentrations ~~across~~ within the two different sample sets, ~~sites~~, measurements at 3T, further described below, were performed on each sample set~~for each experiment~~. After Hct measurement, e~~E~~ight commercially available GBCAs (Table 1) were ~~then~~ serially diluted in human whole blood (Valley Biomedical Products & Services, Inc.). The metal concentration of Gd in the same bottles which were used for dilution, were verified by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES;) after all the experiments(Table 2), this was performed in the basic science labs of Bracco Imaging. An initial preliminary experiment demonstrated that the presence of citrate did not affect GBCA relaxation times, and thus the specimens were diluted with~~collected in~~ sodium citrate added as an anticoagulant. Samples were stored at 4 ± 0.05℃ until the day of scan. The half-life of human serum is approximately 20 days and thus all measurements were preformed less than 10 days within preparation of the whole blood samples~~serum~~.

All solutions were placed into glass NMR tubes (5.5×0.5cm, Wilmad-Lab Glass Company). Both ends were sealed with silicone to prevent evaporation, leakage, and contamination. Each tube was used only once and was disposed of at the end of the measurement. The samples were serially diluted with GBCA to achieve final concentrations of 0, 0.0625, 0.125, 0.25, 0.5, 1, 2, and 4 $mmol \cdot L^{-1}$ GBCA at pH=7.4±0.05. The samples were then loaded into the subsequently described phantom and scanned at 1.5T, 3T, and 7T. To minimize the error due to sedimentation of red blood cells, all the tubes were gently agitated before and during the MR image acquisitions.

Properties of the samples utilized for 1.5 and 3T measurements at site 1 were: total protein concentration of 6.5±0.05g.dL^{-1}, albumin of 4.3±0.05g·dL^{-1}, and Hct of 50±0.5. Properties of the samples utilized for 7T measurements at site 2 were: total protein concentration of 6.3± 0.05g·dL^{-1}, albumin of 4.2±0.05g·dL^{-1}, and Hct of 45±0.5.

~~Additional samples were also prepared for 3T measurements compared to those samples utilized for the 7T measurements at site 2 for quality control purpose~~For quality control purposes both as subset of each of the two sample sets were imaged on a 3T scanner and the results were compared~~s~~. Concentrations utilized for these subset quality control measurements were from 0.25 to 4$mmol \cdot L^{-1}$. ~~To minimize the error due to sedimentation of red blood cells, all the tubes were gently agitated before and during the MR image acquisitions.~~

Custom Built Relaxivity Phantom

All the tubes were placed parallel in a custom-designed acrylic holder in which three plastic screws were utilized to sandwich the tubes, holding them in place between two plastic plates (Fig.1). Each holder contained 2 sets of GBCAs (0.0625~4 $mmol \cdot L^{-1}$) and a tube without any

GBCA as a control. The holder was put in the center of an air tight cylindrical acrylic container with a removable cap. The container was equipped with two equal sized flanges to allow for water to flow in and out and an additional small hole in which a fiber optic probe for temperature measurement was inserted (Site 1: Essential, In vivo, Florida, USA; site 2: Fluor optic temperature probe, LumaSense Technologies, California, USA). To control temperature, plastic tubing was attached to the flanges. The tubing was then connected to a hot water bath (Cole-Parmer Company, Illinois, USA), this allowed for warm water to flow in and out of the phantom. The water exchange inside the phantom was manually controlled to maintain a narrow temperature range for the samples (37±0.5℃). During scan acquisition, the water only circulated outside of the phantom to avoid artifacts from the motion of water.

Imaging protocol

Measurements were performed at 1.5T (Avanto, Siemens Healthcare, Erlangen, Germany) on a clinical whole body MR scanner with an 8-channel knee coil for signal acquisition, a 3T (Skyra, Siemens Healthcare, Erlangen, Germany) MR system with a Tx/Rx 15-Channel knee coil, and a 7T (Philips Healthcare, Cleveland, USA) preclinical MR scanner with a Tx/Rx 32-channel head coil. Additional measurements were performed at 3T (Verio, Siemens Healthcare, Erlangen, Germany) with a Tx/Rx 15-Channel knee coil. For each measurement a single five millimeter thick slice was obtained perpendicular to the long axis of the tubes. The tubes were positioned parallel to B_0 along the z-axis in the magnet.

The longitudinal relaxation rate constant R_1 was calculated as the inverse of T_1 relaxation time. Successive inversion recovery (IR) turbo spin-echo sequences with varying inversion times (TIs)

were used to measure the longitudinal relaxation rate constant R_1.

1) Protocol for 1.5 and 3T

Two different protocols (with different ranges of TI and TR times) aimed at evaluation of different ranges of T_1 times were used in this study. This was done to account for the large range of T_1 times of the dilutions and for the necessity of having a TR time at least 3 times the expected T_1.[11] When looking at the results in retrospect it was deemed unnecessary to perform ~~two~~both protocols, as our results show no statistically significance difference. ~~–~~Nonetheless we chose to report results from both protocols here. ~~based on the choice of a TR at least three times longer than the expected T_1 time[11](11).~~ The 1.5T and 3T ~~Due to the fact that each holder contain 2 sets of GBCAs and one control tube, all~~samples were imaged with both of the following ~~the samples were performed with these two protocols at the same time.~~protocols, aimed at measuring T1 values in parentheses:

Protocol 1 (for assessment of T_1 values from 50~600ms):

TI =30, 60, 90, 120, 150, 250, 400, 600, 800, 1200, 1600, 2000, 2400, 2800, and 3200ms.

TR=1500ms+TI.

Protocol 2 (for assessment of T1 values longer than 600ms):

TI =60, 120, 250, 400, 800, 1600, 2400, 3200, 5000, 7000 and 10000ms.

TR=4000ms+TI.

For both protocols, the following parameters were held constant:

TE=15ms (3T)/11ms (1.5T), matrix=256×256; field of view =80×80mm^2, slice thickness=5mm, and in plane spatial resolution=3.2 pixel/mm.

2) Protocol for 7T

For 7T, the imaging protocol was optimized for the higher field strength. After a preliminary experiment on two of eight GBCAs, specific TRs and TIs were chosen to cover the whole concentration range within a reasonable scan time that allowed for temperature to be maintained, and to maintain image quality equivalent to that at 1.5T and 3T.

Protocol:

TI =60, 80, 120, 160, 220, 350, 500, 650, 900, 1800, 3000, and 5000 ms.

TR=3000ms+TI.

TE=10ms, matrix=320×320; field of view =90×90mm2, slice thickness=5mm, in plane spatial resolution=3.~~556~~ 6 pixel/mm.

Calculation of longitudinal relaxation rate constant R_1

~~Technically R1 is a rate constant, but we will abbreviate it as relaxation rate.~~ Once the images were acquired, regions of interest (ROIs) were automatically placed utilizing custom-designed Matlab code to detect the mean signal intensities (SIs) of the sample within the tubes. The code was designed to only include pixels within the sample and to eliminate partial volume artifact, resulting in ROIs of 100 pixels for 3T/1.5T and 140 pixels for 7T. The MatLab code produced mean ROI measurements as well as an image that showed the ROI placements. All images were manually observed for proper MatLab ROI placement. ROIs that were observed to include

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susceptibility artifact were then manually placed and this measurement was used in subsequent calculations. Since the signal intensities were all positive, because the scanners provided outputs magnitude data only, the inflection point was chosen according to the best fit curve equation (1). Thus positive numbers below the inflection point along the x-axis were converted to negative. R_1 values were obtained using equation (1) and a 3 parameter curve fitting tool (Sigma Plot 12.0). After plotting SIs versus TIs, an exponential curve was constructed to characterize R_1, and the fitting error was used to describe the uncertainty of R_1. (Fig. 2)

$$SI_{TI} = A + B\, e^{-TI \cdot R_1} \quad (1)$$ [12]

The 7T Philips system had a post processing algorithm that scaled the pixel values of each collected MR image. This made the images unacceptable for relaxation measurements and the raw SI values had to be restored. Restoration was accomplished using equation (2), scale slope and intercept is Philips tag included in the DICOM file for each image, and all the intercept were 0 in this study.

$$SI_{7T} = \frac{SI_{Matlab} - Intercept}{Scale\ slope} \quad (2)$$

Calculation of Relaxivities

Relaxivity is the second order rate constant defining the ability of any GBCA to catalyze water proton relaxation[1] defined as the rate at which R_1 changes with a change in GBCA concentration, in other words, the slope of the line obtained by plotting R_1 against the concentrations of contrast agent[1](1).

The r_1 values were calculated from equation (3), where $R_{1(c)}$ was the relaxation rate constant

of the contrast agent at concentration C, and $R_{1(0)}$ denoted the relaxation rate constant of whole blood. Respective error bars were attached to the plot of R_1 versus concentration. The curve fit error was used to estimate the errors of r_1 (Fig. 3).

$$r_1 = \frac{R_{1(c)} - R_{1(0)}}{C} \qquad (3)$$

For 1.5T and 3T evaluations, measurements from two protocols were utilized in three different ways to calculate r_1. This was done to insure that we had the most accurate relaxivity values possible and to insure that the two different protocols did not significantly impact our results.

Method I: r_1 was calculated by data gained from protocol 1 only

Method Ⅱ: r_1 was calculated by combined data from protocol 1 and protocol 2 (concentration range from 0.0625~0.25mmol·L^{-1})

Method Ⅲ: r_1 was calculated by data gained from protocol 1 over the concentration range from 0.5~4 mmol·L^{-1} and from protocol 2 over the concentration range from 0.0625~0.25 mmol·L^{-11}.

Statistical Analysis

For the concentration ranges ~~from~~ 0.25~0.0625mmol·L^{-1} and blood only controls ~~ones~~, ~~r_1~~ longitudinal relaxation rate constant R_1 ~~was~~ ~~which~~ were calculated ~~from~~ using Protocol 1 ~~for all eight GBCAs in human whole blood and was~~ were compared with those calculated from Protocol 2 using a one way repeat measure ANOVA (SigmaPlot 12.0). For those failed with Equal Variance

Test, test execution ended by Ranks. The longitudinal relaxation rate constant R_1 of human whole blood from site 1, which were calculated from protocol 1, were compared with site 2 using a Rank sum test (SigmaPlot 12.0).

Results

ICP-OES results of eight GBCAs were shown in Table 2. All of them were slightly lower than the label.

Comparison between the longitudinal relaxation rate constant R_1 calculations from ~~of~~ two imaging protocols at 1.5T and 3T

At 1.5T, there was no statistically significant difference seen between R_1 values calculated using protocol 1 or 2 ($p > 0.05$) aside from a borderline statistically significant difference found for Gd-DTPA ($p = 0.049$). These results are summarized in Table ~~2~~3. No statistically significant differences between R_1 calculations between the two protocols were found for any contrast agents at 3T ($p>0.1$). These results are summarized in Figure 3 and Table ~~3~~4.

Comparison between the longitudinal relaxation rate constants~~s~~ ~~R_0~~-R_1 of human whole blood at 1.5T, 3T and 7T

The longitudinal relaxation rate constants~~s~~ ~~R_0~~–R_1 of human whole blood were calculated at

~~different~~ the thress field strengths with comparisons of the ~~R_0~~ R_1 values obtained based on the protocol utilized. At both 1.5 and 3T, there were statistically significant differences in calculated ~~R_0~~ R_1 based on the protocol utilized (For 1.5T, p=0.002, for 3T, p=0.027). For each site, four control samples were used. This is shown in Table ~~4~~ 5 and Figure 4. There was no statistically significant difference seen between R_1 values calculated with protocol 1of human whole blood used in the two sites at 3T.

r_1 values of the eight compared GBCAs

The r_1 relaxivities of the eight compared GBCAs were calculated for the three methods at 1.5 and 3T. These values are shown in Table ~~5~~ 6 and Figure 5. An overview of the eight agents and their respective r_1 relaxivities at 1.5, 3, and 7T is presented in Figure 6.

Comparison of r_1 values for the 8 GBCAs diluted in human whole blood with previous results

Table ~~6~~ 7 details results from the present study for measurement of r_1 relaxivities for the 8 GBCAs alongside previous results published in the scientific literature. Based on our calculated standard deviations and the results from all three methods, we present the values in table 7 as our most accurate measurement of r_1 for all GBCAs. These results were obtained using method II.

Discussion

This study calculates the r_1 relaxivities of the eight available GBCAs at 1.5, 3, and 7T from seven different concentrations in human whole blood at 37℃. Relaxivity values represent the GBCAs' ability to shorten T_1 times. GBCAs with high relaxivity values shorten tissue T_1 times more efficaciously. GBCA's are divided in terms of their ability to bind serum proteins into the nonprotein-binding GBCAs (Gd-DTPA, Gd-DOTA, Gd-HP-DO3A, Gd-BT-DO3A, Gd-DTPA-BMA and Gd-DTPA-BMEA), the weakly protein-binding GBCAs (Gd-BOPTA, Gd-EOB-DTPA), and the strongly protein-binding Gd agent (MS-325)[13]~~(13)~~. All GBCAs approved in the United States and Europe other than MS-325 are assessed in the present work. In doing so, limitations of several previous studies are addressed, as variations in experimental methods result in variations in calculated r_1 values. Although several previous works have been performed on this topic, none of them perform calculations in human whole blood. In the study by Rohrer[8]~~(8)~~ et al, all available MR contrast agents were compared at 0.47T, 1.5T, 3T and 4.7T at 37℃, but only two concentrations (0.25 and 0.5 mmol/L) were utilized for the calculation. Further, MR contrast agents were diluted in water, bovine plasma, and canine blood to measure relaxivities, rather than whole human blood. Pintaske[3]~~(3)~~ et al performed a similar comparison in human plasma and over a wider concentration range (0.01 to 64mmol/L) at 0.2T, 1.5T and 3T. However, only three GBCAs (Gd-DTPA, Gd-BT-DO3A, and Gd-BOPTA) were evaluated. The Noebauer-Huhmann[14]~~(14)~~ et al study was the first performed at 7T. In this work, T_1 relaxivity values of eight GBCAs commercially available in Europe at 3T and 7T were compared at 37℃.However, only four different concentrations were utilized, and error estimates were not provided.

The aforementioned previous studies have likewise not attempted to obtain baseline T_1 values

of whole human blood at various field strengths. T_1 values of blood may be influenced by hematocrit fractions (Hct), temperature, PH, and oxygenation levels[9, 15-19]~~(9, 15-19)~~. In fact, accurate measurements of human blood T_1 values is an important topic in itself, as these values are an important MR parameter for quantitative physiological and functional MR measurements, such as arterial spin labeling (ASL) with cerebral blood volume (CBV) calculations. Previously, T_1 values of bovine blood had been measured at 1.5T, 3T[18]~~(18)~~, 4.7T, 7T, and 9.4T[17]~~(17)~~. T_1 values of rat blood have also been measured at 11.7T[16]~~(16)~~. Human blood T_1 values have also recently been measured at 3T[19]~~(19)~~ and 7T[15]~~(15)~~.

Inversion recovery (IR) turbo spin-echo pulses have traditionally been the standard pulse sequences by which to measure longitudinal relaxation rate constants~~s~~ (R_1). In the present study, two protocols were utilized at 1.5T and 3T. These protocols incorporated different T1 times based on principles outlined by Ogg et al[11]~~(11)~~ – namely, that TR should be at least three times longer than the T_1 time. Data points generated by plotting SI against TI with protocol 2 yielded data points evenly distributed along the exponential curve, whereas few data points measured with protocol 1 were distributed on the flat part of the curve (Fig.3). Theoretically, the data from protocol 2 should give a more accurate depiction of relaxation curves for the lower concentrations, thus leading to more accurate results. Although results from two protocols were slightly different from each other, no statistically significant differences were found between them, except for borderline statistically significant results with Gd-DTPA at 1.5T (Table 2). The measurements of the longitudinal relaxation rate constants~~s~~ of human whole blood from the two protocols were statistically significantly different both at 1.5T and 3T (Table 4). Theoretically, the results from protocol 2 should be more accurate than those from protocol 1 as the former gave a relaxation

curve fitting the expected exponential distribution more closely (Fig.4). The statistically significant differences in the R_1 measurements for whole blood are likely due to the long T_1 relaxation time of whole blood and the fact that one of the protocols utilized a TR=1500 ms. ~~–~~In the end the difference was not large enough to impact our results. We found no statistical difference when using results from protocol 1 or 2 when calculating relaxivity.

Only one protocol was utilized to measure whole blood longitudinal relaxation at 7T. The calculated T_1 values of human whole blood in the present study was in accordance with the study done by Rane et al[15]~~(15)~~ (2.29±0.1s for human arterial blood, 2.09±0.12s for human venous blood, 2.16±0.1s for clinic patient's venous blood). Also T_1 values of human blood in the present study were linearly dependent on B_0, which ~~was~~ is consistent with previous studies[15-18]~~(15-18)~~ (Fig. 4).

According to the literature, r_1 is characterized by the change in relaxation rate constant per unit concentration of Gd chelates and is independent of concentration. The number of relaxation rate constant data points collected is critical to derive accurate statistical predictions from the regression analysis. Only two concentrations (0.25 and 0.50mmol·L^{-1}) were utilized for curve computation by Rohrer et al[8]~~(8)~~, and four concentrations (0.25, 0.5, 1, and 2 mmol·L^{-1}) by Noebauer-Huhmann et al[14]~~(14)~~. In the present study, 7 different concentrations were utilized. Slight differences are also found when obtaining relaxation rate constant measurements with the two utilized protocols. Theoretically, R_1 measured with protocol 2 should be more accurate for lower GBCA concentrations and protocol 1 for higher GBCA concentrations. Thus, it is reasonable to assume that calculations ~~which~~ that combine data obtained using the two different protocols are best utilized to include~~account for~~ the wide range of concentrations present. Considering this issue~~To account for this~~, three different methods were used to calculate r_1. ~~All~~

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tThe results were similar for all three approaches (Table 56). For all eight GBCAs, results calculated with method II, which incorporated combined results from protocol 1 and protocol 2, The calculated error (standard deviation) found from method II (which incorporated combined results from protocol 1 and protocol 2) was less than or equal to those calculated with the other methods, thus we present this as our most accurate results and they are shown in Table 7.demonstrated standard deviations less than or equal to those calculated with the other methods. In Noebauer-Huhmann et al[14], the small number of concentrations utilized for the r_1 calculation adds to the uncertainty of the results. Pintaske et al[3](3) utilized a wider range of concentrations (0.01, 0.02, 0.03, 0.06, 0.13, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64mmol·L^{-1}) in human blood plasma, however only with three GBCAs. Pintaske et al stated the relationship between R_1 and concentration was nonlinear with Gd-BOPTA. Noebauer-Huhmann et al[14] determined that all 8 GBCAs had nonlinear relationship between R_1 and concentration at both 3T and 7T. In the present study, plots of R_1 against concentration of eight GBCAs showed no consistent pattern and were not definitely linear or nonlinear (Fig. 5). Different serum total protein and albumin in each of experimental material setups could be the reason[20, 21](20, 21). Another reason for discrepancies can be attributed to inaccuracies of concentration determination and /or species-dependent influentences of the blood plasma samples.

One potential limitation of the current study is that blood oxygenation may influence blood T_1 values. However, conflicting conclusions regarding the influence of blood oxygen content have been reached in previous studies[15-19](15-19). In Lin et al[16](16), rat blood T_1 values were found to be independent of oxygenation. The study of Rane et al[15](15) demonstrated that T_1 values of human blood at 7T differed depending on whether arterial or venous blood was examined. Other

limitations include the fact that citrate was present in the~~was not removed from~~ blood in the present study, as discussed above in the methods section. However, a preliminary experiment demonstrated no differences in relaxivity values on this basis, thus justifying this approach. Also~~Furthermore~~, different sources of blood were utilized, a fact ~~which~~ that was accounted for by controlling for hematocrit fractions and ensuring that serum total protein and albumin were similar. In addition, 3T measurements were obtained on samples of both the blood sources to further ensure standardization. The relaxivity values acquired at 3T from both sites/samples were similar. Lower Gd concentration than stated on the label may also have affected our results, however the relaxivity calculations reflect the formulation of the agents as commercially supplied (Table 2).

The current study provides T_1 relaxivity measurements, obtained from human whole blood, comparing the eight gadolinium chelates commonly employed world-wide as intravenous contrast media for magnetic resonance, at current relevant field strengths (1.5, 3, and 7T) and physiologic temperature. Statistically significant differences are noted both between agents, and between field strengths. The results from the present study should serve as a reasonable and reliable reference for further clinical utilization.

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Figures legends

Figure 1 Custom-built holder and phantom

All the tubes are placed parallel to each other in a custom-design acrylic holder, using plastic screws to sandwich the tubes and hold them in place. The holder was placed in the center of phantom with a removable cap. There was one hole in the center in which to place a temperature probe (*). The holder is shown in longitudinal (A) and transverse (B) views.

Figure 2 Analysis of data

(A) Regions of interests (ROIs) were automatically placed by the MATLAB program, signified by ~~yellow~~ white rings (@3T, TI=60, zoomed in the right corner). Pixels inside the rings were measured. The temperature probe is denoted by the ~~yellow~~ arrow.

(B) Mean SI values were utilized to plot T_1 relaxation curves. This particular example shows curves for Gd-EOB-DTPA at a concentration of 0.25 m~~mol·L^{-1}~~M and 4mM at 3T.

~~C~~(C) Plotting R_1 against concentrations to calculate the r_1 (at 3T for Gd-EOB-DTPA).

Figure 3 Graph illustrating SIs versus TIs of two protocols utilized to measure r_1 relaxivity at 1.5T and 3T. This example is of Gd-DOTA evaluated at a concentration of 0.0625mmol·L^{-1} at 1.5T, with protocol 1 and 2 (A) ~~and 2 (B)~~, and at 3T with protocol 1 ~~(C),~~ and 2 (~~D~~B).

Figure 4 Calculation of human whole blood R_1 relaxation rate constant as performed using protocol 1 (A) and 2 (B) at 1.5T, as well as protocol 1 and 2 at 3T. Four control samples were included in the calculation.The calculated values were linearly dependent on B_0(C).

Figure 5 Illustrated is R_1 plotted against concentration for the 8 GBCAs at 1.5T, 3T and 7T.

Figure 6 An –overview of the eight agents and their respective r1 relaxivities at 1.5, 3, and 7T is presented, in a graphical format.

Tables

Table 1 Investigated Gd-based MR contrast agents

Chemical name	Generic Name (INN)	Trade name	Manufacturer
Gd-BOPTA	Gadobenate dimeglumine	MultiHance	Bracco
Gd-DO3A-butrol	Gadobutrol	Gadovist/Gadavist	Bayer HealthCare
Gd-DOTA	Gadoterate meglumine	Dotarem	Guerbet
Gd-DTPA	Gadopentetate dimeglumine	Magnevist	Bayer HealthCare
Gd-DTPA-BMA	Gadodiamide	Omniscan	GE-Healthcare
Gd-DTPA-BMEA	Gadoversetamide	OptiMARK	Covidien
Gd-EOB-DTPA	Gadoxetic acid disodium	Primovist/Eovist	Bayer HealthCare
Gd-HP-DO3A	Gadoteridol	ProHance	Bracco

*In alphabetic order INN, international Nonproprietary Names

Table 2 ICP-OES results of Investigated Gd-based MR contrast agents

Chemical name	Formulation (M)	ICP-OES (M)	Percentage variation form reference value
Gd-BOPTA	0.5	0.47±0.01	-5.81
Gd-DO3A-butrol	1	0.95±0.03	-4.91
Gd-DOTA	0.5	0.48±0.01	-4.64
Gd-DTPA	0.5	0.48±0.01	-3.61
Gd-DTPA-BMA	0.5	0.49±0.01	-2.33
Gd-DTPA-BMEA	0.5	0.47±0.00	-5.23
Gd-EOB-DTPA	0.25	0.23±0.00	-6.56
Gd-HP-DO3A	0.5	0.48±0.01	-3.84

Table 2 3 Comparison of R_1 (s^{-1}) of GBCAs using two protocols at 1.5T

	Protocol 1			Protocol 2			*F*	*P*
Concentration (mmol.L^{-1}M)	0.25	0.125	0.0625	0.25	0.125	0.0625		
Gd-BOPTA	2.53±0.09	1.71±0.07	1.18±0.04	2.52±0.06	1.65±0.07	1.18±0.03	2.335	0.266
Gd-DO3A-butrol	2.04±0.07	1.30±0.05	0.98±0.05	2.01±0.08	1.30±0.05	1.01±0.02	0.008	0.936
Gd-DOTA	1.77±0.06	1.16±0.05	0.93±0.08	1.79±0.12	1.17±0.05	0.93±0.03	2.133	0.282
Gd-DTPA	2.19±0.06	1.43±0.05	1.08±0.04	2.22±0.11	1.44±0.08	1.11±0.03	19.104	0.049
Gd-DTPA-BMA	1.91±0.04	1.34±0.05	1.01±0.05	1.97±0.07	1.34±0.06	1.02±0.03	2.292	0.269
Gd-DTPA-BMEA	2.06±0.05	1.34±0.05	0.99±0.05	2.02±0.05	1.34±0.04	0.99±0.03	0.650	0.505
Gd-EOB-DTPA	2.48±0.07	1.69±0.08	1.15±0.05	2.42±0.06	1.65±0.04	1.14±0.03	5.957	0.135
Gd-HP-DO3A	2.31±0.06	1.70±0.07	1.36±0.05	2.32±0.05	1.65±0.07	1.34±0.05	1.570	0.337

Table 3 4 Comparison of R_1 (s s^{-1}) of GBCAs using two protocols at 3T

	Protocol 1			Protocol 2			*F*	*P*
Concentration(m mol.L $^{-1}$ M)	0.25	0.125	0.0625	0.25	0.125	0.0625		
Gd-BOPTA	2.06±0.02	1.36±0.01	0.97±0.03	2.33±0.64	1.32±0.07	0.96±0.02	0.531	0.542
Gd-DO3A-butrol	1.83±0.01	1.16±0.03	0.84±0.04	1.72±0.15	1.14±0.03	0.84±0.05		1*
Gd-DOTA	1.61±0.07	1.05±0.03	0.82±0.03	1.51±0.06	1.03±0.02	0.82±0.03	2.589	0.249
Gd-DTPA	1.78±0.02	1.26±0.04	0.96±0.02	1.75±0.07	1.23±0.05	0.98±0.03	0.895	0.444
Gd-DTPA-BMA	1.71±0.01	1.19±0.03	0.91±0.03	1.67±0.10	1.17±0.05	0.90±0.02	9.318	0.093
Gd-DTPA-BMEA	1.86±0.02	1.20±0.03	0.86±0.03	1.87±0.11	1.19±0.03	0.87±0.03	0.0717	0.814
Gd-EOB-DTPA	2.08±0.05	1.41±0.03	0.92±0.11	2.15±0.14	1.36±0.01	0.95±0.03	0.153	0.733
Gd-HP-DO3A	1.75±0.02	1.51±0.02	1.18±0.04	1.64±0.10	1.51±0.05	1.19±0.03	0.791	0.468

*Equal Variance Test failed, test execution ended by Ranks

Table 4—5 Longitudinal Relaxation Rates R_0 R_1 (s^{-1}) of human whole blood at 37℃

1.5T$^{\$}$		3T$^{\&}$		7T	
Protocol 1	Protocol 2	Protocol 1*		Protocol 2	
0.74±0.004	0.78±0.00801	0.59±0.05	0.56±0.0103#	0.64±0.05	0.44±0.00701#

Blood from second resourceof site 2

Comparison of the results of two protocols: $ F=107.824,P=0.002; & F= 16.352, P=0.027

Comparison of results of two sites:* t=0.715 ,P=0.514

Table ~~5~~ 6 Comparison of r_1 (~~$L \cdot s^{-1} \cdot mmol^{-1}$~~ mM^{-1}) of GBCAs with two protocols at 1.5T and 3T

	1.5T			3T		
	I	II	III	I	II	III
Gd-BOPTA	6.15±0.38	6.20±0.36	6.16±0.39	5.36±0.33	5.37±0.33	5.33±0.37
Gd-DO3A-butrol	4.58±0.19	4.61±0.18	4.58±0.18	4.42±0.26	4.46±0.24	4.44±0.27
Gd-DOTA	3.89±0.14	3.91±0.13	3.89±0.14	3.40±0.31	3.43±0.29	3.41±0.32
Gd-DTPA	4.22±0.34	4.25±0.32	4.21±0.34	3.75±0.18	3.76±0.17	3.75±0.19
Gd-DTPA-BMA	4.46±0.09	4.47±0.08	4.46±0.08	3.87±0.16	3.89±0.15	3.88±0.17
Gd-DTPA-BMEA	4.41±0.19	4.43±0.18	4.41±0.19	4.23±0.12	4.24±0.12	4.23±0.12
Gd-EOB-DTPA	7.24±0.17	7.24±0.15	7.25±0.17	5.42±0.27	5.45±0.26	5.42±0.27
Gd-HP-DO3A	4.32±0.49	4.39±0.47	4.32±0.50	3.41±0.49	3.46±0.46	3.41±0.51

Method Ⅰ r_1 was calculated by data gained from protocol 1 only

Method Ⅱ r_1 was calculated by data gained from protocol 1 and protocol 2 (concentration range from 0.0625~0.25 ~~mmol.L~~$^{-1}$mM)

Method Ⅲ r_1 was calculated by data gained from protocol 1(concentration range from 0.5~4mmol.L^{-1}) and protocol 2 (concentration range from 0.0625~0.25 m~~mol.L~~$^{-1}$M).

Table ~~6~~ 7 Comparison of r_1 relaxivities ($s^{-1} \cdot mM^{-1}$) of GBCAs in different scenarios

Author	Solvent	Temperature	Field strength (T)	Gd-BOPTA	Gd-DO3A-butrol	Gd-DOTA	Gd-DTPA	Gd-DTPA-BMA	Gd-DTPA-BMEA	Gd-EOB-DTPA	Gd-HP-DO3A
~~Rohrer[4]~~Rohrer[8]	Bovine plasma	37℃	1.5	6.3(6.0-6.6)	5.2(4.9-5.5)	3.6(3.4-3.8)	4.1(3.9-4.3)	4.3(4.0-4.6)	4.7(4.4-5.0)	6.9(6.5-7.3)	4.1(3.9-4.3)
	Canine whole blood	37℃	1.5	6.7(6.3-7.1)	5.3(5.0-5.6)	4.2(3.9-4.5)	4.3(4.0-4.6)	4.6(4.3-4.9)	5.2(4.9-5.5)	7.3(6.9-7.7)	4.4(4.1-4.7)
	Bovine plasma	37℃	3	5.5(5.2-5.8)	5.0(4.7-5.3)	3.5(3.3-3.7)	3.7(3.5-3.9)	4.0(3.8-4.2)	4.5(4.2-4.8)	6.2(5.9-6.5)	3.7(3.5-3.9)
~~Pintaske[3]~~Pintaske3	Human plasma	37℃	1.5	~~8.1~~7.9±0.4	4.7±0.2		3.9±0.2				
	Human plasma	37℃	3	~~6.3~~5.9±0.4	~~3.6~~4.5±0.2		~~3.3~~3.9±0.2				
Noebauer-~~Huhmann[5]~~Huhmann14	Human plasma	37℃	3	5.10	4.90	3.30	3.50	3.60		5.4	3.50
	Human plasma	37℃	7	4.30	4.70	3.20	3.30	3.50		4.8	3.30
Present Study	Human whole blood*	37℃	1.5	6.20±0.36	4.61±0.18	3.91±0.13	4.25±0.32	4.47±0.08	4.43±0.18	7.24±0.15	4.39±0.47
	Human whole blood*	37℃	3	5.37±0.33	4.46±0.24	3.43±0.29	3.76±0.17	3.89±0.15	4.24±0.12	5.45±0.26	3.46±0.46
	Human whole blood#	37℃	3	5.00±0.13	4.32±0.10	3.19±0.40	3.34±0.19	3.86±0.17	3.95±0.05	5.34±0.90	3.41±0.16
	Human whole blood	37℃	7	4.67±0.09	4.20±0.24	2.82±0.40	3.11±0.36	3.72±0.19	4.26±0.21	4.90±0.08	3.35±0.12

* Results calculated with two protocols (Method II)

#Concentration range from 0.25~4 mmol/LM

Figure1A
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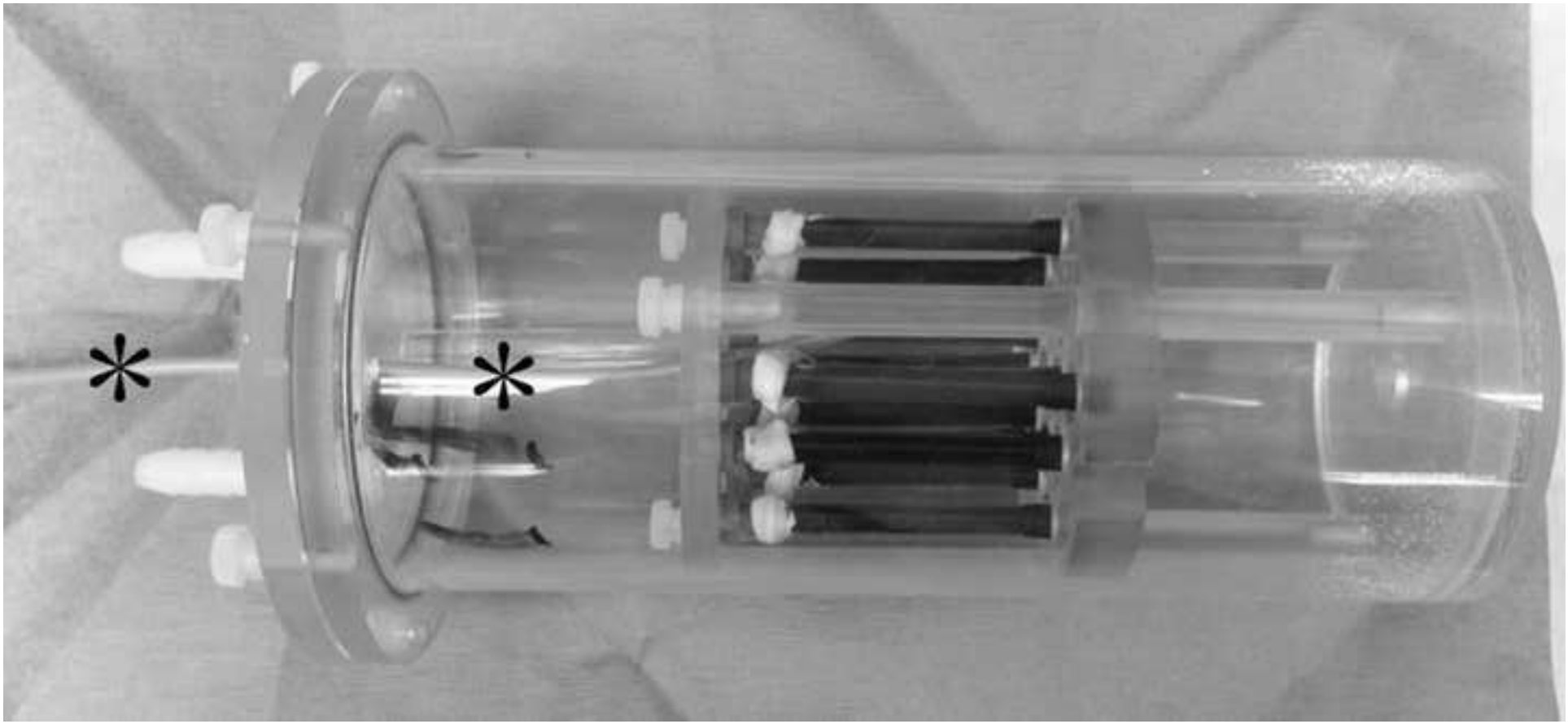

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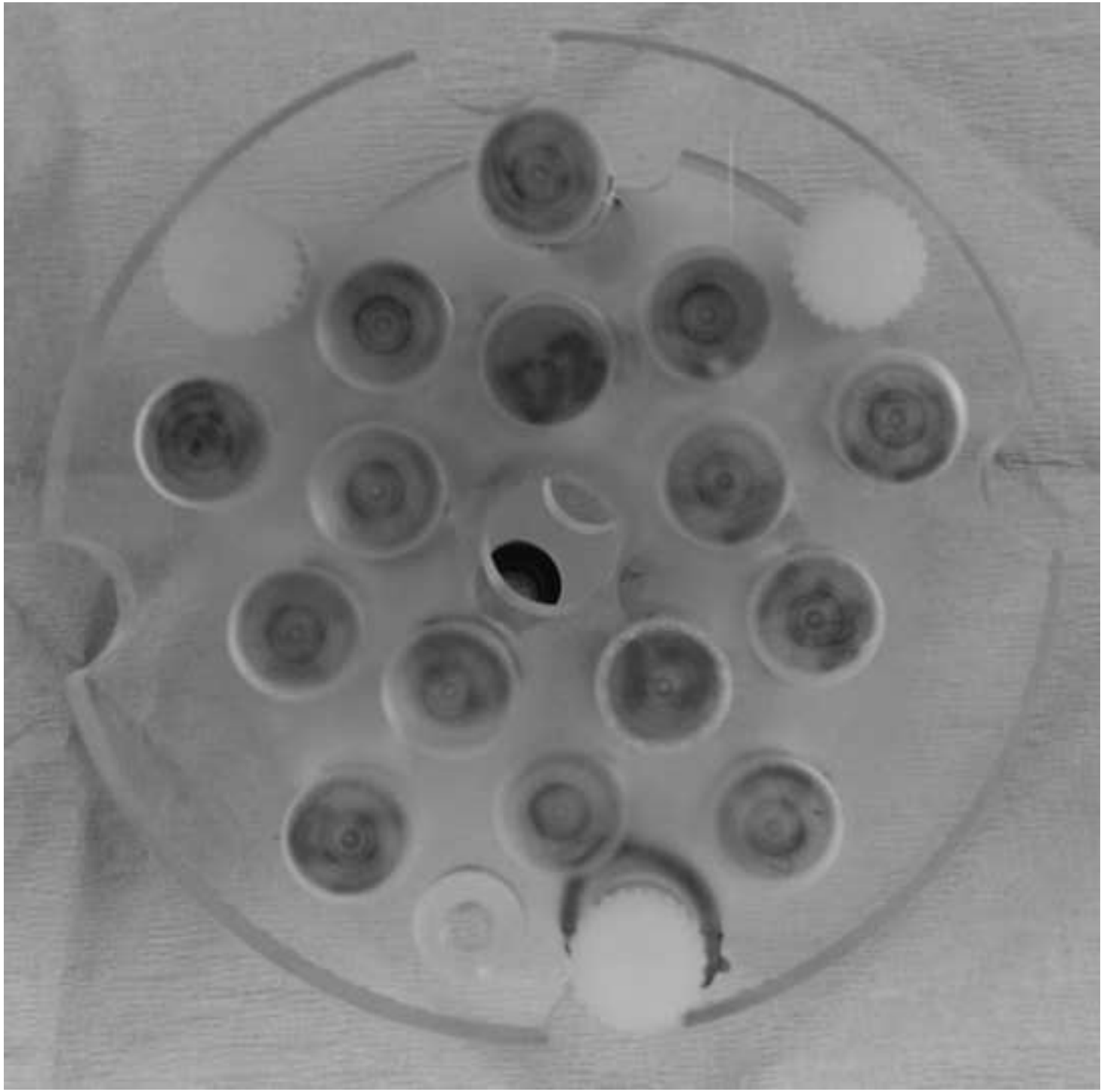

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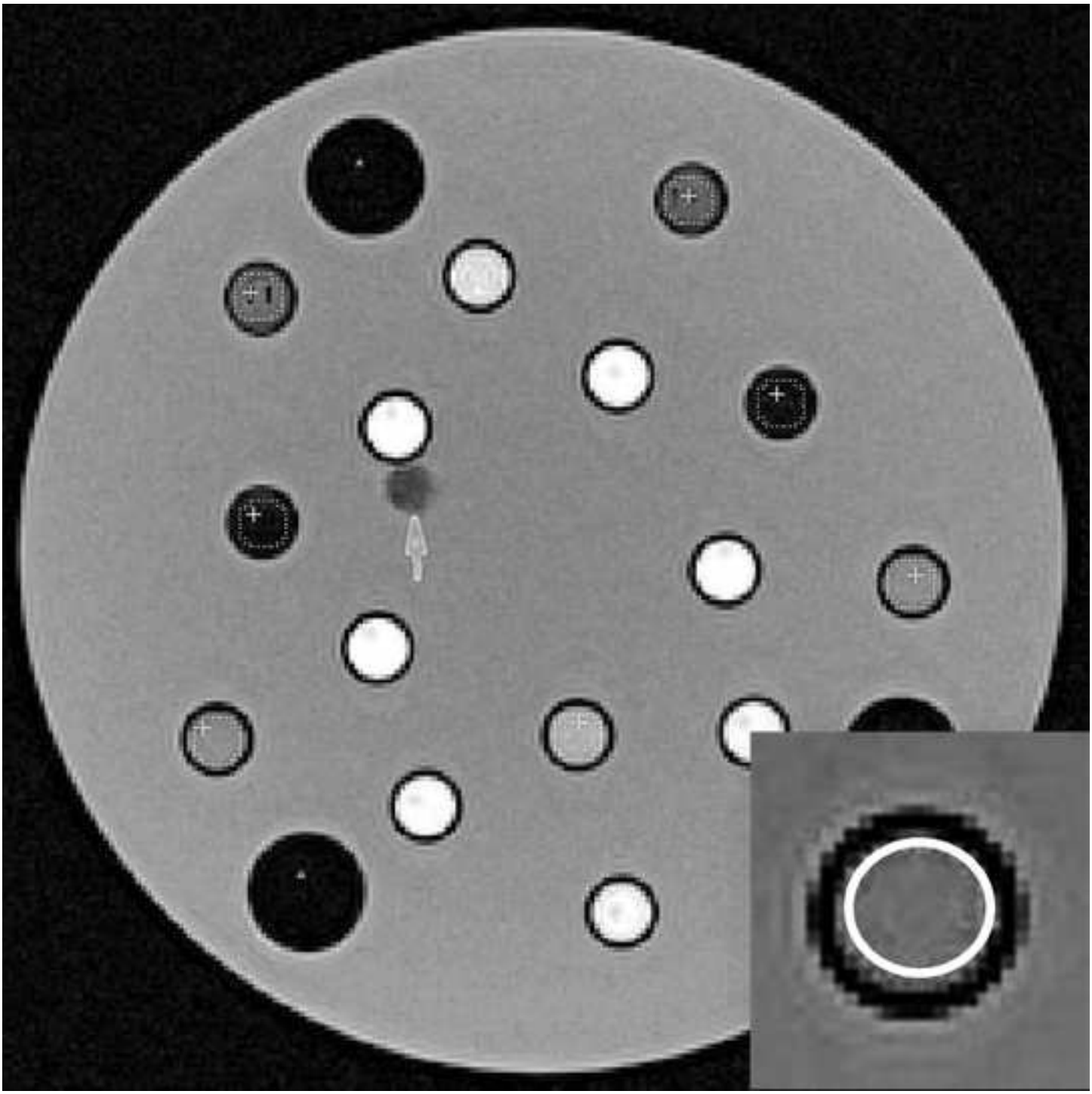

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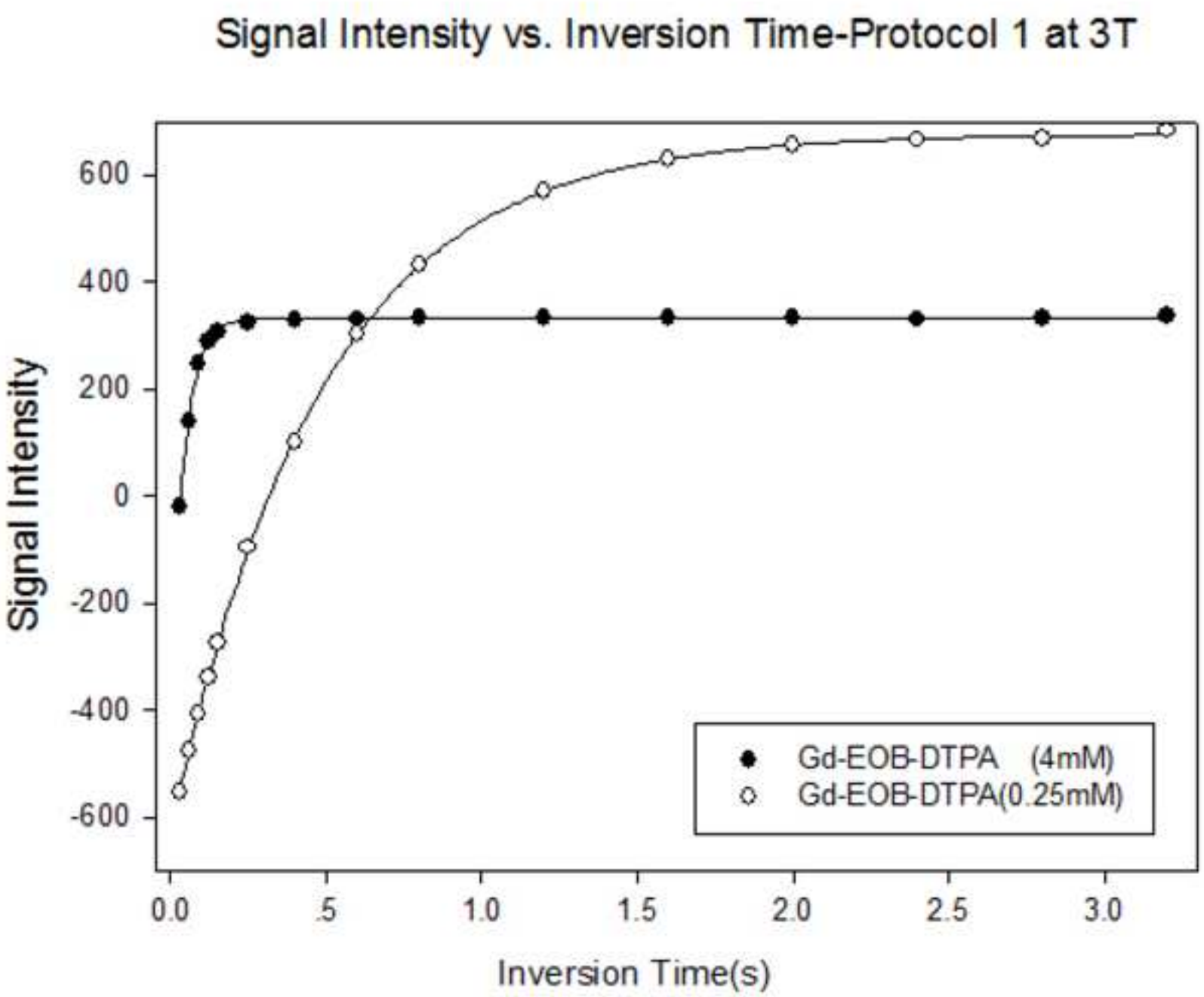

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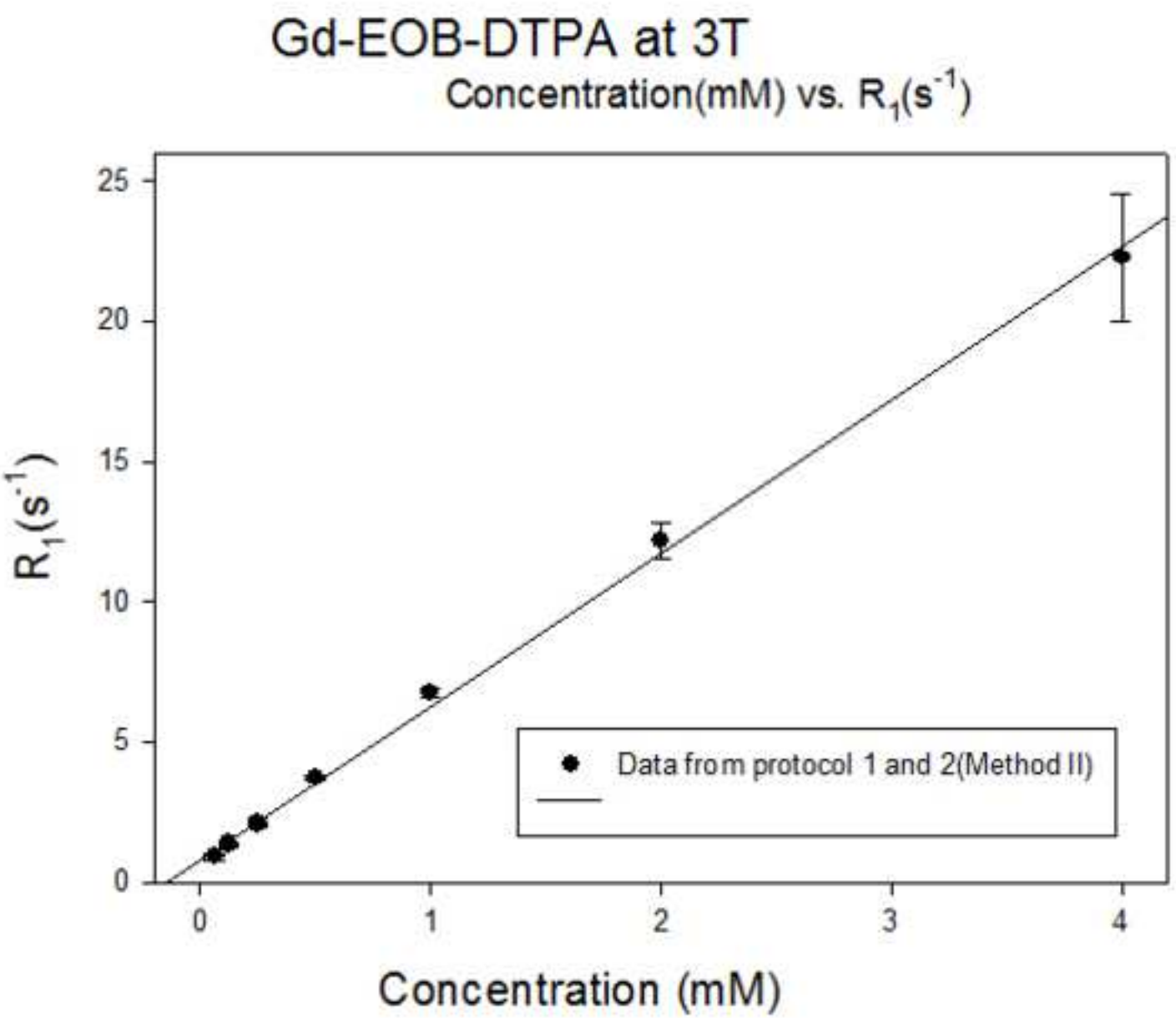

Figure3A

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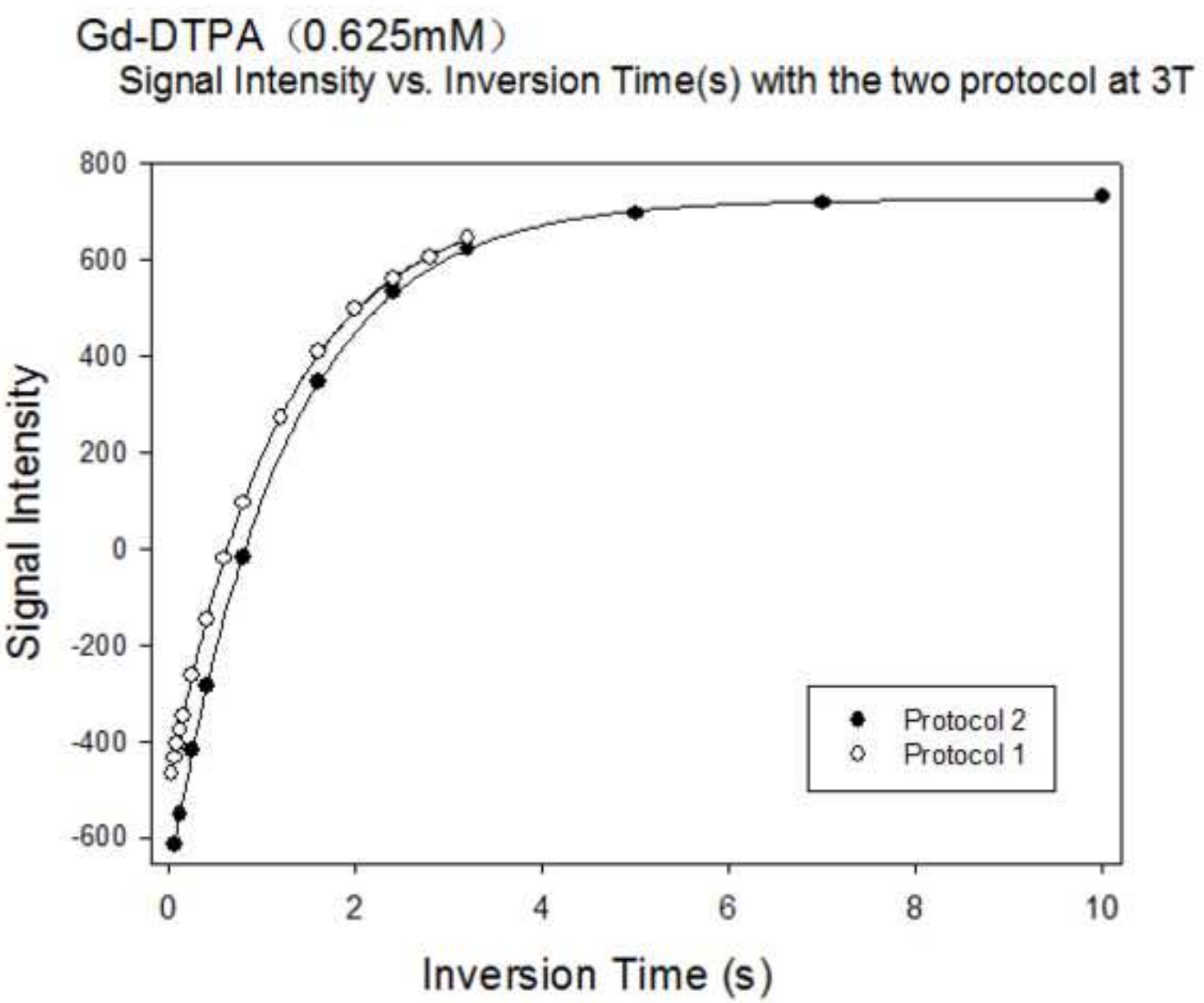

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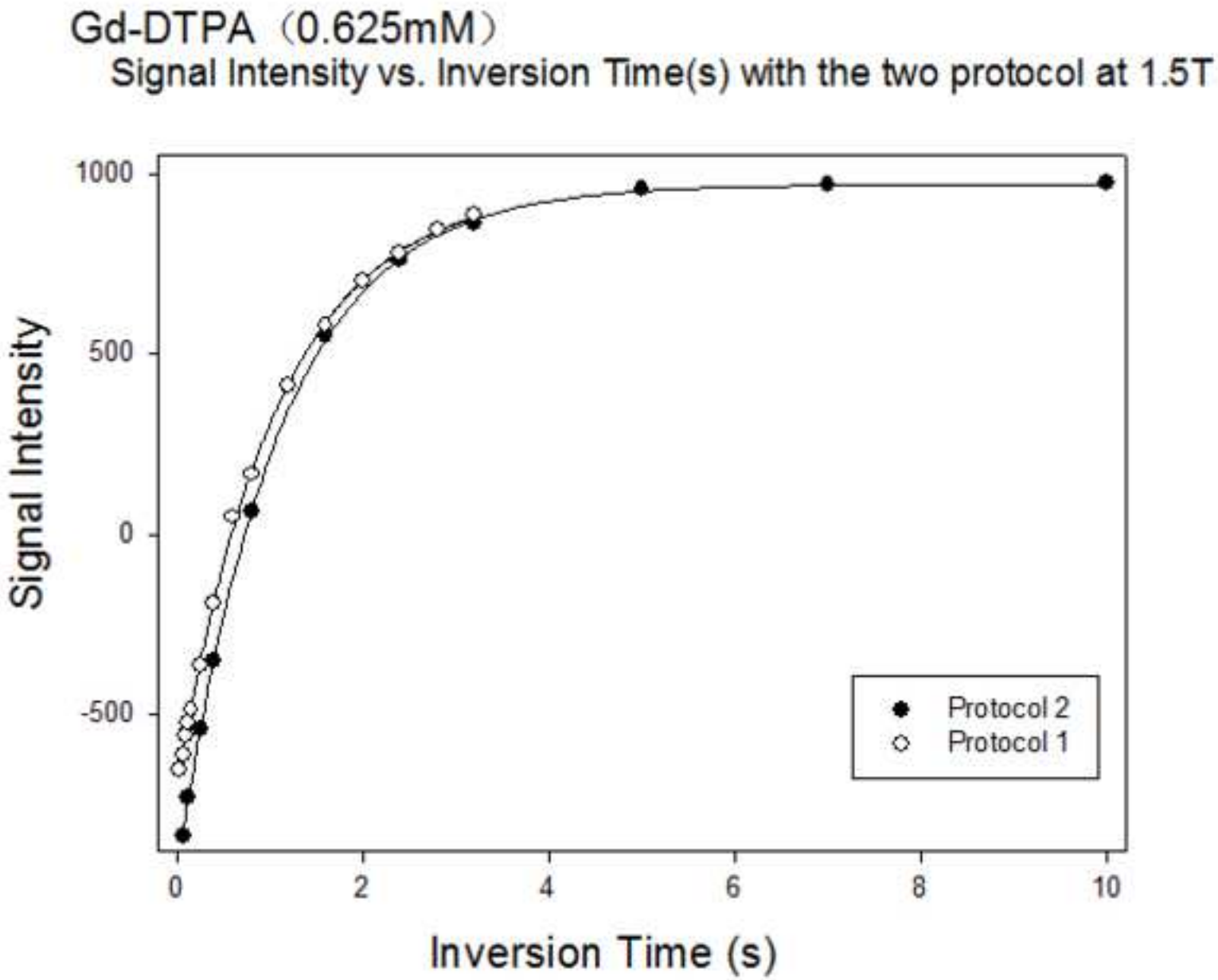

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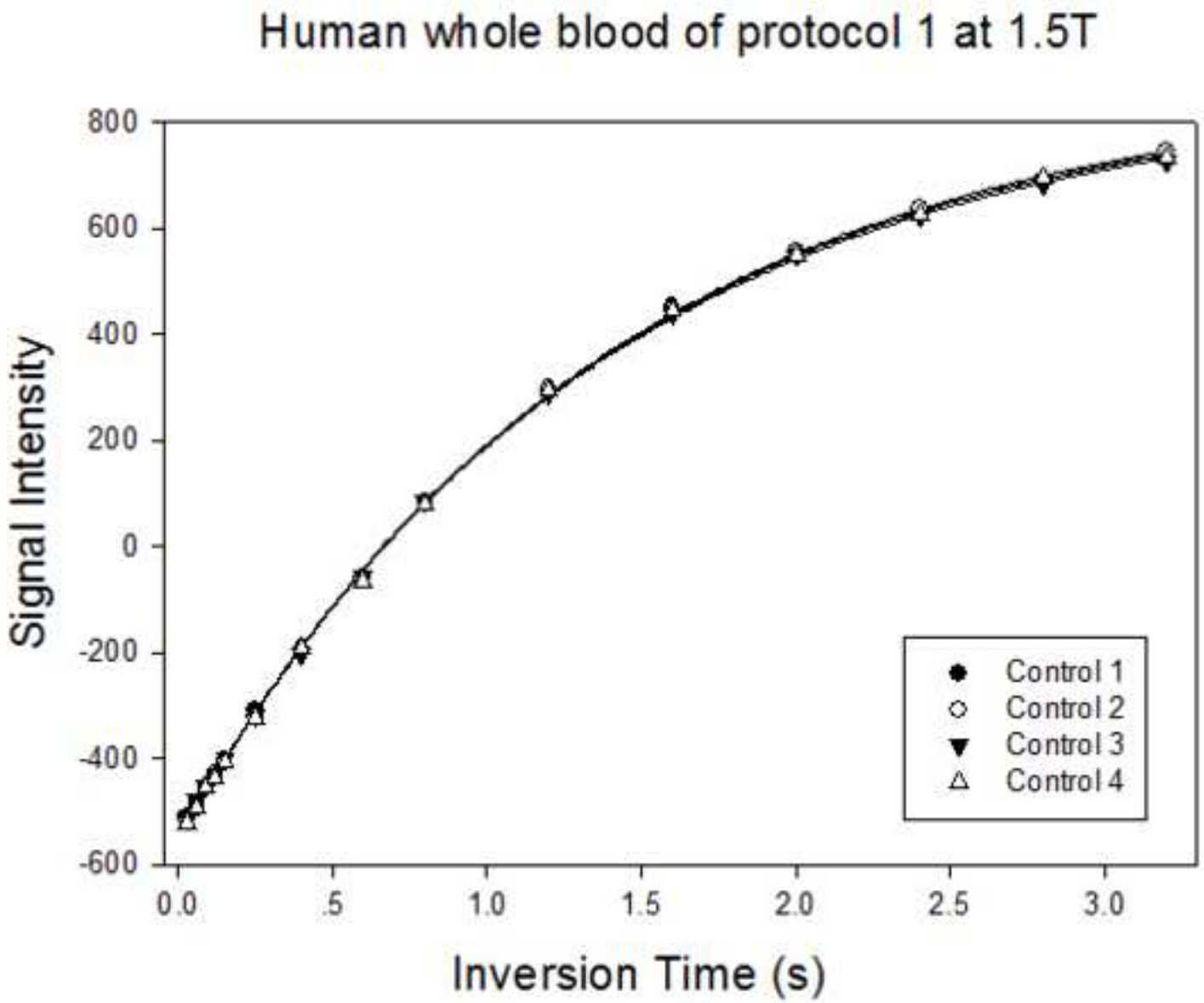

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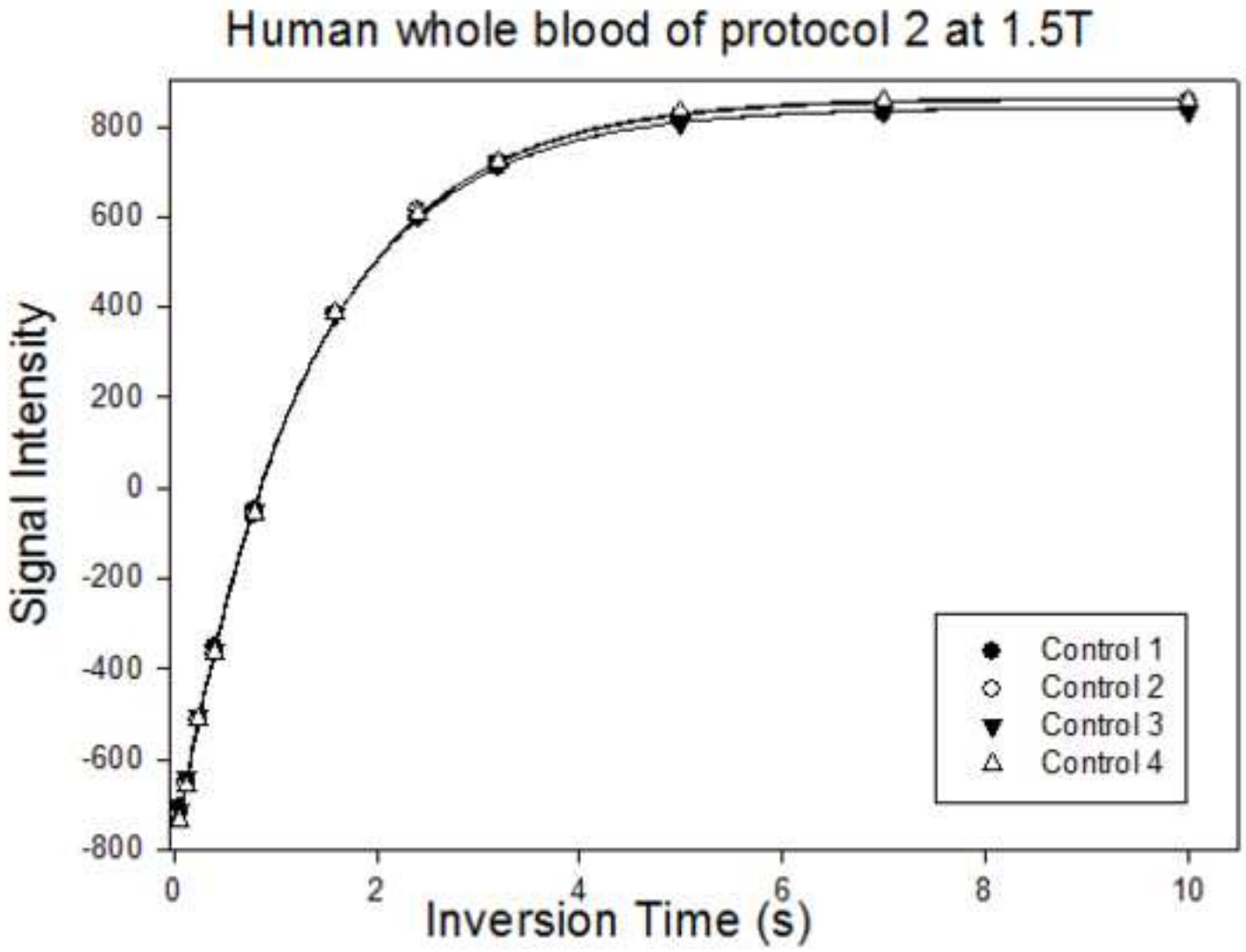

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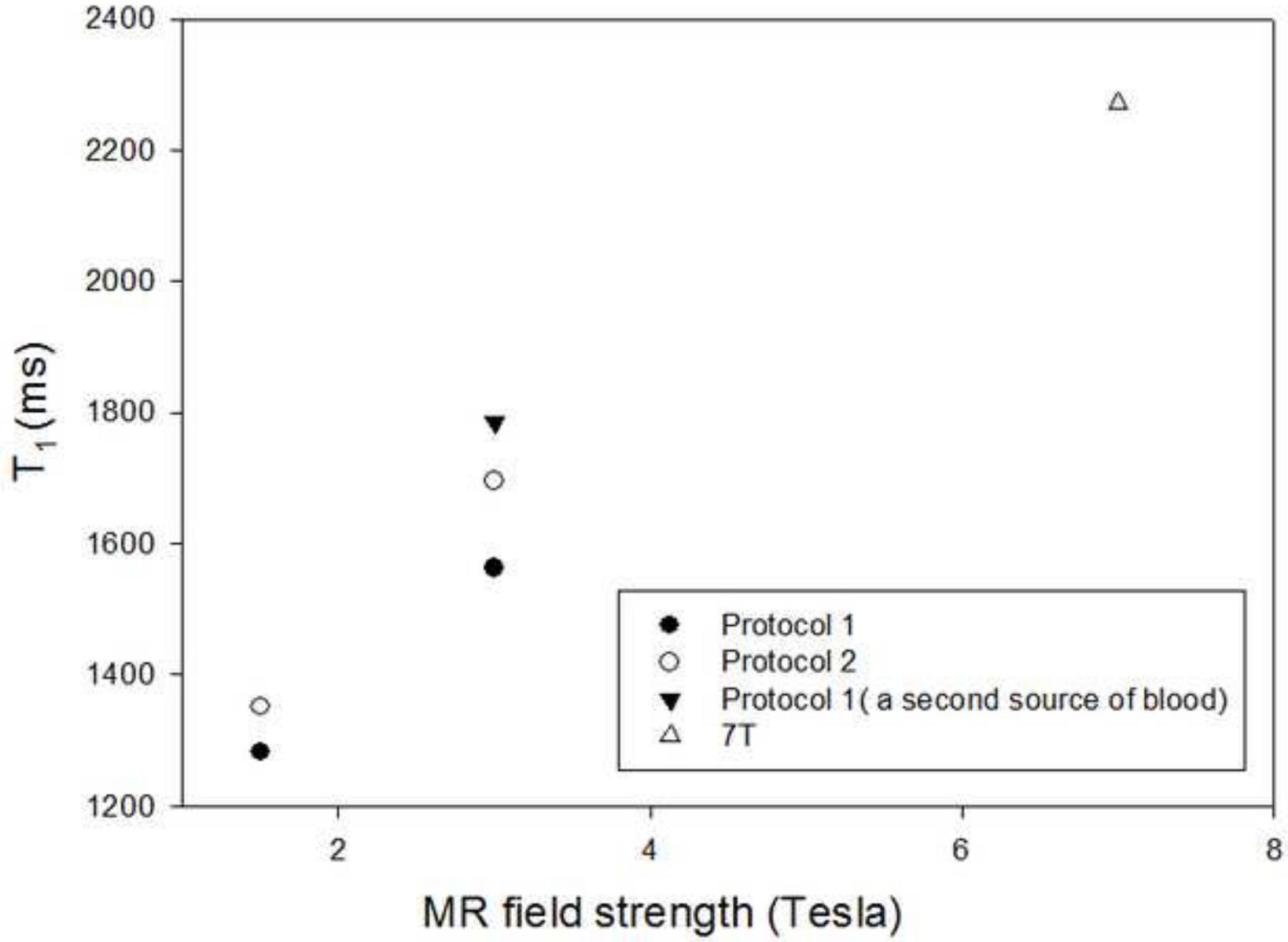

Figure5

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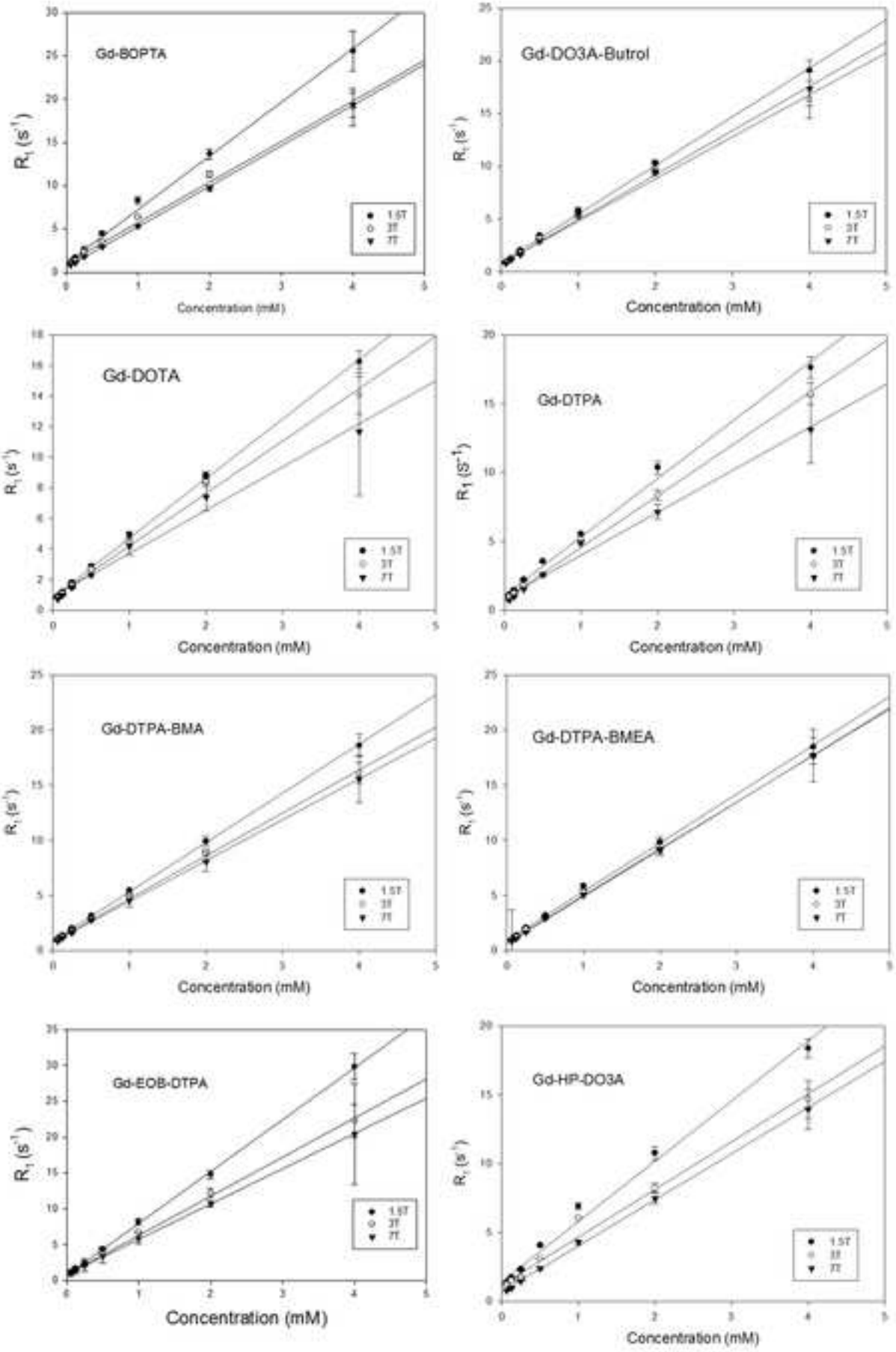

Figure6
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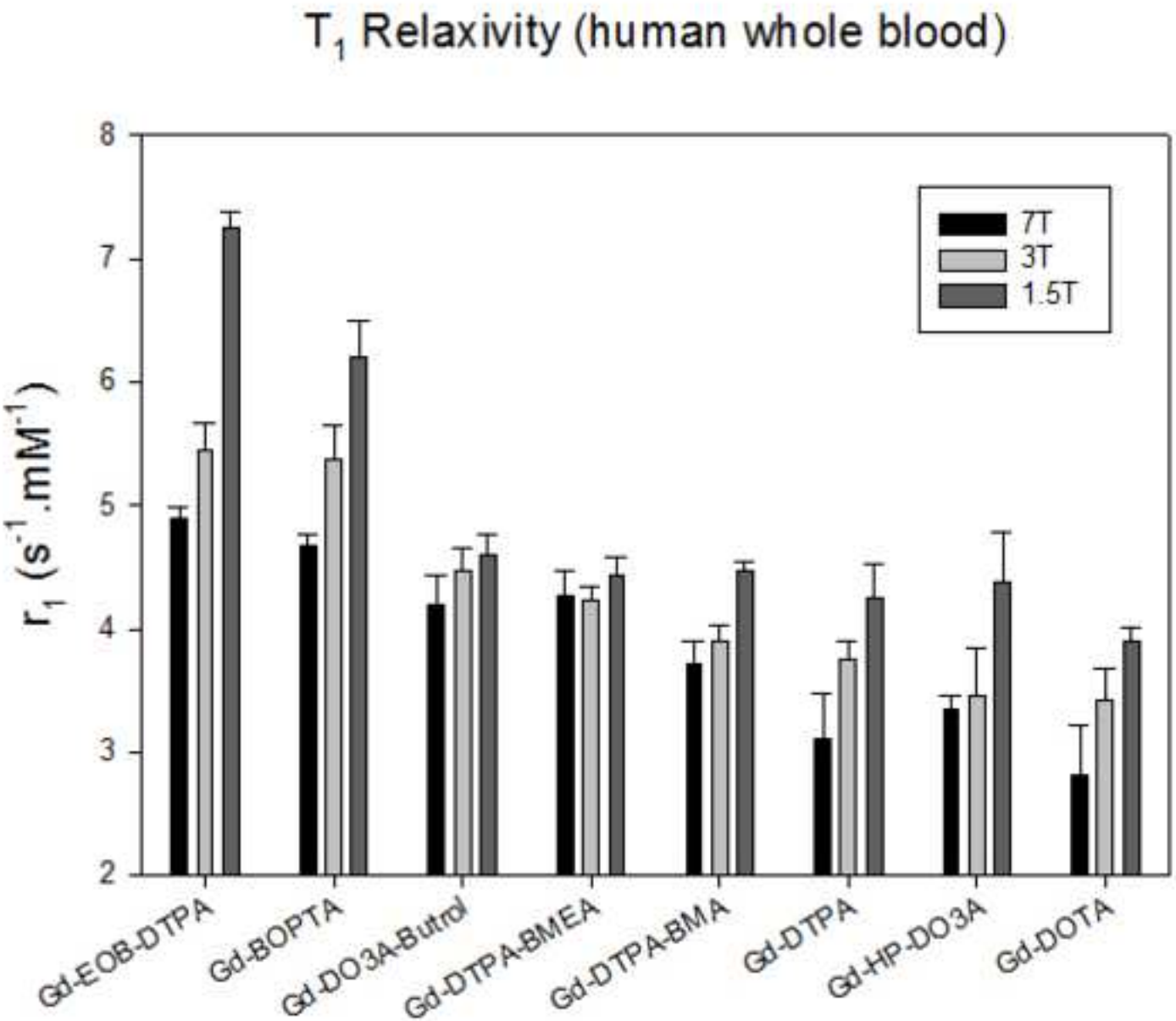

T_1 relaxivities of Gadolinium-Based Magnetic Resonance Contrast Agents in Human Whole Blood at 1.5, 3 and 7 Tesla

Abstract

Objectives Calculation of accurate T_1 relaxivity (r_1) values for gadolinium based MR contrast agents (GBCAs) is a complex process. As such, often referenced r_1 values for the GBCAs at 1.5T, 3T, and 7T are based on measurements obtained in media that are not clinically relevant, derived from only a small number of concentrations, or available for only a limited number of GBCAs. This study derives the r_1 values of the eight commercially available GBCAs in human whole blood at 1.5T, 3T and 7T.

Materials and Methods Eight GBCAs were serially diluted in human whole blood, at 7 concentrations from 0.0625 to 4mmol/L. A custom-built phantom held the dilutions in air tight cylindrical tubes maintained at 37±0.5℃ by a heat circulating system. Images were acquired utilizing inversion recovery sequences with TIs from 30ms to 10s at 1.5, 3T, and 60ms to 0.5s at 7T. A custom Matlab program was used to automate signal intensity measurements from images acquired of the phantom. SigmaPlot was used to calculate T_1 relaxation times and finally r_1.

Results Measured r_1 values in units of $L \cdot mmol^{-1}s^{-1}$ at 1.5T(3T/7T) were 3.9±0.2 (3.4±0.4/2.8±0.4) for Gd-DOTA, 4.6±0.2 (4.5±0.3/4.2±0.3) for Gd-DO3A-butrol, 4.3±0.4 (3.8±0.2/3.1±0.4) for Gd-DTPA, 6.2±0.5 (5.4±0.3/4.7±0.1) for Gd-BOPTA, 4.5±0.1 (3.9±0.2/3.7±0.2) for Gd-DTPA-BMA, 4.4±0.2 (4.2±0.2/4.3±0.2) for Gd-DTPA-BMEA, 7.2±0.2 (5.5±0.3/4.9±0.1) for Gd-EOB-DTPA, and 4.4±0.6 (3.5±0.6/3.4±0.1) for Gd-HP-DO3A. The agents can be stratified by relaxivity, with a significant additional dependency on field strength.

Conclusions This report quantifies for the first time T1 relaxivity for all eight gadolinium chelates in common clinical use worldwide, at current relevant field strengths, in human whole blood at physiological temperature (37℃). The measured r_1 values differ to a small degree from previously published values, where such comparisons exist, with the current r_1 measurements being that most relevant to clinical practice. The macrocyclic agents, with the exception of Gd-DO3A-butrol, have slightly lower r_1 values when compared to the two much less stable linear agents, Gd-DTPA-BMA and Gd-DTPA-BMEA. The two agents with hepatobiliary excretion, Gd-EOB-DTPA and Gd-BOPTA, have at 1.5 and 3T substantially higher r_1 values than all other agents.

Key words: relaxivity; MRI contrast media; gadolinium; whole blood; field strength dependence; 1.5T; 3T; 7T comparative studies

Introduction

Gadopentetate dimeglumine (Magnevist, Gd-DTPA) was the first gadolinium-based MR contrast agent (GBCA) approved for clinical use, which occurred in 1988. To date, nine GBCAs have been approved for use in Europe and the United States.[1, 2] The additional eight include Gd-BOPTA (gadobenate dimeglumine, MultiHance), Gd-DO3A-Butrol (gadobutrol, Gadovist/Gadavist), Gd-DOTA (gadoterate meglumine, Dotarem), Gd-DTPA-BMA (gadodiamide, Omniscan), Gd-DTPA-BMEA (gadoversetamide, OptiMARK), Gd-EOB-DTPA (gadoxetic acid disodium, Primovist/Eovist), Gd-HP-DO3A (gadoteridol, ProHance), and MS-325 (gadofosveset trisodium, Ablavar). In clinical practice, the GBCAs are most commonly utilized to improve detection and differentiation of pathological lesions and to visualize the vasculature in magnetic resonance angiography applications.

Relaxivity is a critical parameter in determining the relative efficacy of the GBCAs. Relaxivity is defined as the change in reciprocal relaxation time per unit concentration of Gd chelate. T_1 relaxivity, known as r_1, is influenced by many variables including magnetic field strength, temperature, environmental conditions, and protein concentration.[1, 3-5]

Previous in vitro studies have examined the relative relaxivities of the gadolinium chelates. However, these have been incomplete—not including all relevant field strengths or deriving calculations from a wide range of concentrations—or inapplicable to human imaging—not performed in human whole blood under at physiologic temperatures, or failing to account for nonlinear 1/T1 vs [Gd] when proteins are present in the protein binding agents..[3, 4, 6-10] This study

aims to address these limitations by determining the T_1 relaxivity values across a wide range of concentrations at 1.5T, 3T, and 7T for the eight primary, commercially available, GBCAs (excluding MS-325, which is strongly protein bound, seldom used in clinical work in most of the world and no longer commercially available in Europe) in human whole blood at 37℃.

Materials and Methods

Preparation of human whole blood samples

Whole human blood was acquired from a commercial supplier (Valley Biomedical Products & Services, Inc.). Due to 7 Tesla scanners not being widely available, the experiments were performed at two different sites—one housing the 1.5 and 3 Tesla scanners (site 1) and the other housing the 7 Tesla scanner (site 2). This also required a different human blood sample to be utilized at each respective site because it was not possible to preserve the first sample set for use at the second site. For standardization purposes, hematocrit fractions (Hct) of each sample set were measured prior to dilution (site 1: Hct was 50±0.5, site 2: Hct was 45±0.5). To ensure relaxivity results were not affected by the different Hct concentrations within the two different sample sets, , measurements at 3T, further described below, were performed on each sample set. After Hct measurement, eight commercially available GBCAs (Table 1) were serially diluted in human whole blood (Valley Biomedical Products & Services, Inc.). The metal concentration of Gd in the same bottles which were used for dilution, were verified by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES;) after all the experiments(Table 2), this was performed in the basic science labs of Bracco Imaging. An initial preliminary experiment demonstrated that the presence of citrate did not affect GBCA relaxation times, and thus the specimens were diluted with sodium citrate added as an anticoagulant. Samples were stored at 4±0.05℃ until the day of scan. The half-life of human serum is approximately 20 days and thus all measurements were preformed less than 10 days within preparation of the whole blood samples.

All solutions were placed into glass NMR tubes (5.5×0.5cm, Wilmad-Lab Glass Company). Both

ends were sealed with silicone to prevent evaporation, leakage, and contamination. Each tube was used only once and was disposed of at the end of the measurement. The samples were serially diluted with GBCA to achieve final concentrations of 0, 0.0625, 0.125, 0.25, 0.5, 1, 2, and 4 $mmol \cdot L^{-1}$ GBCA at pH=7.4±0.05. The samples were then loaded into the subsequently described phantom and scanned at 1.5T, 3T, and 7T. To minimize the error due to sedimentation of red blood cells, all the tubes were gently agitated before and during the MR image acquisitions.

Properties of the samples utilized for 1.5 and 3T measurements at site 1 were: total protein concentration of 6.5±0.05g.dL^{-1}, albumin of 4.3±0.05g$\cdot dL^{-1}$, and Hct of 50±0.5. Properties of the samples utilized for 7T measurements at site 2 were: total protein concentration of 6.3± 0.05g$\cdot dL^{-1}$, albumin of 4.2±0.05g$\cdot dL^{-1}$, and Hct of 45±0.5.

For quality control purposes both as subset of each of the two sample sets were imaged on a 3T scanner and the results were compared. Concentrations utilized for these subset quality control measurements were from 0.25 to 4$mmol \cdot L^{-1}$.

Custom Built Relaxivity Phantom

All the tubes were placed parallel in a custom-designed acrylic holder in which three plastic screws were utilized to sandwich the tubes, holding them in place between two plastic plates (Fig.1). Each holder contained 2 sets of GBCAs (0.0625~4 $mmol \cdot L^{-1}$) and a tube without any GBCA as a control. The holder was put in the center of an air tight cylindrical acrylic container with a removable cap. The container was equipped with two equal sized flanges to allow for water to flow in and out and an additional small hole in which a fiber optic probe for temperature measurement was inserted (Site 1: Essential, In vivo, Florida, USA; site 2: Fluor optic temperature

probe, LumaSense Technologies, California, USA). To control temperature, plastic tubing was attached to the flanges. The tubing was then connected to a hot water bath (Cole-Parmer Company, Illinois, USA), this allowed for warm water to flow in and out of the phantom. The water exchange inside the phantom was manually controlled to maintain a narrow temperature range for the samples (37±0.5℃). During scan acquisition, the water only circulated outside of the phantom to avoid artifacts from the motion of water.

Imaging protocol

Measurements were performed at 1.5T (Avanto, Siemens Healthcare, Erlangen, Germany) on a clinical whole body MR scanner with an 8-channel knee coil for signal acquisition, a 3T (Skyra, Siemens Healthcare, Erlangen, Germany) MR system with a Tx/Rx 15-Channel knee coil, and a 7T (Philips Healthcare, Cleveland, USA) preclinical MR scanner with a Tx/Rx 32-channel head coil. Additional measurements were performed at 3T (Verio, Siemens Healthcare, Erlangen, Germany) with a Tx/Rx 15-Channel knee coil. For each measurement a single five millimeter thick slice was obtained perpendicular to the long axis of the tubes. The tubes were positioned parallel to B_0 along the z-axis in the magnet.

The longitudinal relaxation rate constant R_1 was calculated as the inverse of T_1 relaxation time. Successive inversion recovery (IR) turbo spin-echo sequences with varying inversion times (TIs) were used to measure the longitudinal relaxation rate constant R_1.

1) Protocol for 1.5 and 3T

Two different protocols (with different ranges of TI and TR times) aimed at evaluation of different ranges of T_1 times were used in this study. This was done to account for the large range

of T_1 times of the dilutions and for the necessity of having a TR time at least 3 times the expected T_1.[11] When looking at the results in retrospect it was deemed unnecessary to perform both protocols, as our results show no statistically significance difference. Nonetheless we chose to report results from both protocols here. [11]. The 1.5T and 3T samples were imaged with both of the following protocols, aimed at measuring T1 values in parentheses:

Protocol 1 (for assessment of T_1 values from 50~600ms):

TI =30, 60, 90, 120, 150, 250, 400, 600, 800, 1200, 1600, 2000, 2400, 2800, and 3200ms.

TR=1500ms+TI.

Protocol 2 (for assessment of T1 values longer than 600ms):

TI =60, 120, 250, 400, 800, 1600, 2400, 3200, 5000, 7000 and 10000ms.

TR=4000ms+TI.

For both protocols, the following parameters were held constant:

TE=15ms (3T)/11ms (1.5T), matrix=256×256; field of view =80×80mm^2, slice thickness=5mm, and in plane spatial resolution=3.2 pixel/mm.

2) Protocol for 7T

For 7T, the imaging protocol was optimized for the higher field strength. After a preliminary experiment on two of eight GBCAs, specific TRs and TIs were chosen to cover the whole concentration range within a reasonable scan time that allowed for temperature to be maintained, and to maintain image quality equivalent to that at 1.5T and 3T.

Protocol:

TI =60, 80, 120, 160, 220, 350, 500, 650, 900, 1800, 3000, and 5000 ms.

TR=3000ms+TI.

TE=10ms, matrix=320×320; field of view =90×90mm2, slice thickness=5mm, in plane spatial resolution=3.6 pixel/mm.

Calculation of longitudinal relaxation rate constant R_1

Once the images were acquired, regions of interest (ROIs) were automatically placed utilizing custom-designed Matlab code to detect the mean signal intensities (SIs) of the sample within the tubes. The code was designed to only include pixels within the sample and to eliminate partial volume artifact, resulting in ROIs of 100 pixels for 3T/1.5T and 140 pixels for 7T. The MatLab code produced mean ROI measurements as well as an image that showed the ROI placements. All images were manually observed for proper MatLab ROI placement. ROIs that were observed to include susceptibility artifact were then manually placed and this measurement was used in subsequent calculations. Since the signal intensities were all positive, because the scans provided magnitude data only, the inflection point was chosen according to the best fit curve equation (1). Thus positive numbers below the inflection point along the x-axis were converted to negative. R_1 values were obtained using equation (1) and a 3 parameter curve fitting tool (Sigma Plot 12.0). After plotting SIs versus TIs, an exponential curve was constructed to characterize R_1, and the fitting error was used to describe the uncertainty of R_1. (Fig. 2)

$$SI_{TI} = A + \mathrm{B}\, e^{-TI \cdot R_1} \quad (1)$$ [12]

The 7T Philips system had a post processing algorithm that scaled the pixel values of each collected MR image. This made the images unacceptable for relaxation measurements and the raw SI values had to be restored. Restoration was accomplished using equation (2), scale slope and intercept is Philips tag included in the DICOM file for each image, and all the intercept were 0 in this study.

$$SI_{7T} = \frac{SI_{Matlab} - Intercept}{Scale\ slope} \qquad (2)$$

Calculation of Relaxivities

Relaxivity is the second order rate constant defining the ability of any GBCA to catalyze water proton relaxation[1 1].

The r_1 values were calculated from equation (3), where $R_{1(c)}$ was the relaxation rate constant of the contrast agent at concentration C, and $R_{1(0)}$ denoted the relaxation rate constant of whole blood. Respective error bars were attached to the plot of R_1 versus concentration. The curve fit error was used to estimate the errors of r_1 (Fig. 3).

$$r_1 = \frac{R_{1(c)} - R_{1(0)}}{C} \qquad (3)$$

For 1.5T and 3T evaluations, measurements from two protocols were utilized in three different ways to calculate r_1. This was done to insure that we had the most accurate relaxivity values possible and to insure that the two different protocols did not significantly impact our results.

Method I: r_1 was calculated by data gained from protocol 1 only

Method Ⅱ: r_1 was calculated by combined data from protocol 1 and protocol 2 (concentration range from 0.0625~0.25mmol·L^{-1})

Method Ⅲ: r_1 was calculated by data gained from protocol 1 over the concentration range from 0.5~4 mmol·L^{-1} and from protocol 2 over the concentration range from 0.0625~0.25 mmol·L^{-11}.

Statistical Analysis

For the concentration ranges 0.25~0.0625mmol·L^{-1} and blood only controls, longitudinal relaxation rate constant R_1 were calculated using Protocol 1 were compared with those calculated from Protocol 2 using a one way repeat measure ANOVA (SigmaPlot 12.0). For those failed with Equal Variance Test, test execution ended by Ranks. The longitudinal relaxation rate constant R_1 of human whole blood from site 1, which were calculated from protocol 1, were compared with site 2 using a Rank sum test (SigmaPlot 12.0).

Results

ICP-OES results of eight GBCAs were shown in Table 2. All of them were slightly lower than the label.

Comparison between the longitudinal relaxation rate constant R_1 calculations from two imaging protocols at 1.5T and 3T

At 1.5T, there was no statistically significant difference seen between R_1 values calculated using

protocol 1 or 2 (p> 0.05) aside from a borderline statistically significant difference found for Gd-DTPA (p = 0.049). These results are summarized in Table 3. No statistically significant differences between R_1 calculations between the two protocols were found for any contrast agents at 3T (p>0.1). These results are summarized in Figure 3 and Table 4.

Comparison between the longitudinal relaxation rate constants R_1 of human whole blood at 1.5T, 3T and 7T

The longitudinal relaxation rate constants R_1 of human whole blood were calculated at the thress field strengths with comparisons of the R_1 values obtained based on the protocol utilized. At both 1.5 and 3T, there were statistically significant differences in calculated R_1 based on the protocol utilized (For 1.5T, p=0.002, for 3T, p=0.027). For each site, four control samples were used. This is shown in Table 5 and Figure 4. There was no statistically significant difference seen between R_1 values calculated with protocol 1of human whole blood used in the two sites at 3T.

r_1 values of the eight compared GBCAs

The r_1 relaxivities of the eight compared GBCAs were calculated for the three methods at 1.5 and 3T. These values are shown in Table 6 and Figure 5. An overview of the eight agents and their respective r_1 relaxivities at 1.5, 3, and 7T is presented in Figure 6.

Comparison of r_1 values for the 8 GBCAs diluted in human whole blood with previous results

Table 7 details results from the present study for measurement of r_1 relaxivities for the 8 GBCAs alongside previous results published in the scientific literature. Based on our calculated standard

deviations and the results from all three methods, we present the values in table 7 as our most accurate measurement of r_1 for all GBCAs. These results were obtained using method II.

Discussion

This study calculates the r_1 relaxivities of the eight available GBCAs at 1.5, 3, and 7T from seven different concentrations in human whole blood at 37℃. Relaxivity values represent the GBCAs' ability to shorten T_1 times. GBCAs with high relaxivity values shorten tissue T_1 times more efficaciously. GBCA's are divided in terms of their ability to bind serum proteins into the nonprotein-binding GBCAs (Gd-DTPA, Gd-DOTA, Gd-HP-DO3A, Gd-BT-DO3A, Gd-DTPA-BMA and Gd-DTPA-BMEA), the weakly protein-binding GBCAs (Gd-BOPTA, Gd-EOB-DTPA), and the strongly protein-binding Gd agent (MS-325)[13]. All GBCAs approved in the United States and Europe other than MS-325 are assessed in the present work. In doing so, limitations of several previous studies are addressed, as variations in experimental methods result in variations in calculated r_1 values. Although several previous works have been performed on this topic, none of them perform calculations in human whole blood. In the study by Rohrer[8] et al, all available MR contrast agents were compared at 0.47T, 1.5T, 3T and 4.7T at 37℃, but only two concentrations (0.25 and 0.5 mmol/L) were utilized for the calculation. Further, MR contrast agents were diluted in water, bovine plasma, and canine blood to measure relaxivities, rather than whole human blood. Pintaske[3] et al performed a similar comparison in human plasma and over a wider concentration range (0.01 to 64mmol/L) at 0.2T, 1.5T and 3T. However, only three GBCAs (Gd-DTPA, Gd-BT-DO3A, and Gd-BOPTA) were evaluated. The Noebauer-Huhmann[14] et al study was the first performed at 7T. In this work, T_1 relaxivity values of eight GBCAs commercially available in Europe at 3T and 7T were compared at 37℃.However, only four different concentrations were utilized, and error estimates were not provided.

The aforementioned previous studies have likewise not attempted to obtain baseline T_1 values

of whole human blood at various field strengths. T_1 values of blood may be influenced by hematocrit fractions (Hct), temperature, PH, and oxygenation levels[9, 15-19]. In fact, accurate measurements of human blood T_1 values is an important topic in itself, as these values are an important MR parameter for quantitative physiological and functional MR measurements, such as arterial spin labeling (ASL) with cerebral blood volume (CBV) calculations. Previously, T_1 values of bovine blood had been measured at 1.5T, 3T[18], 4.7T, 7T, and 9.4T[17]. T_1 values of rat blood have also been measured at 11.7T[16]. Human blood T_1 values have also recently been measured at 3T[19] and 7T[15].

Inversion recovery (IR) turbo spin-echo pulses have traditionally been the standard pulse sequences by which to measure longitudinal relaxation rate constants (R_1). In the present study, two protocols were utilized at 1.5T and 3T. These protocols incorporated different T1 times based on principles outlined by Ogg et al[11] – namely, that TR should be at least three times longer than the T_1 time. Data points generated by plotting SI against TI with protocol 2 yielded data points evenly distributed along the exponential curve, whereas few data points measured with protocol 1 were distributed on the flat part of the curve (Fig.3). Theoretically, the data from protocol 2 should give a more accurate depiction of relaxation curves for the lower concentrations, thus leading to more accurate results. Although results from two protocols were slightly different from each other, no statistically significant differences were found between them, except for borderline statistically significant results with Gd-DTPA at 1.5T (Table 2). The measurements of the longitudinal relaxation rate constants of human whole blood from the two protocols were statistically significantly different both at 1.5T and 3T (Table 4). Theoretically, the results from protocol 2 should be more accurate than those from protocol 1 as the former gave a relaxation curve fitting

the expected exponential distribution more closely (Fig.4). The statistically significant differences in the R_1 measurements for whole blood are likely due to the long T_1 relaxation time of whole blood and the fact that one of the protocols utilized a TR=1500 ms. In the end the difference was not large enough to impact our results. We found no statistical difference when using results from protocol 1 or 2 when calculating relaxivity.

Only one protocol was utilized to measure whole blood longitudinal relaxation at 7T. The calculated T_1 values of human whole blood in the present study was in accordance with the study done by Rane et al[15] (2.29±0.1s for human arterial blood, 2.09±0.12s for human venous blood, 2.16±0.1s for clinic patient's venous blood). Also T_1 values of human blood in the present study were linearly dependent on B_0, which is consistent with previous studies[15-18] (Fig. 4).

According to the literature, r_1 is characterized by the change in relaxation rate constant per unit concentration of Gd chelates and is independent of concentration. The number of relaxation rate constant data points collected is critical to derive accurate statistical predictions from the regression analysis. Only two concentrations (0.25 and 0.50mmol·L^{-1}) were utilized for curve computation by Rohrer et al[8], and four concentrations (0.25, 0.5, 1, and 2 mmol·L^{-1}) by Noebauer-Huhmann et al[14]. In the present study, 7 different concentrations were utilized. Slight differences are also found when obtaining relaxation rate constant measurements with the two utilized protocols. Theoretically, R_1 measured with protocol 2 should be more accurate for lower GBCA concentrations and protocol 1 for higher GBCA concentrations. Thus, it is reasonable to assume that calculations that combine data obtained using the two different protocols are best utilized to include the wide range of concentrations present. Considering this issue, three different methods were used to calculate r_1. The results were similar for all three approaches (Table 6). The

calculated error (standard deviation) found from method II (which incorporated combined results from protocol 1 and protocol 2) was less than or equal to those calculated with the other methods, thus we present this as our most accurate results and they are shown in Table 7. In Noebauer-Huhmann et al[14], the small number of concentrations utilized for the r_1 calculation adds to the uncertainty of the results. Pintaske et al[3] utilized a wider range of concentrations (0.01, 0.02, 0.03, 0.06, 0.13, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64mmol·L^{-1}) in human blood plasma, however only with three GBCAs. Pintaske et al stated the relationship between R_1 and concentration was nonlinear with Gd-BOPTA. Noebauer-Huhmann et al[14] determined that all 8 GBCAs had nonlinear relationship between R_1 and concentration at both 3T and 7T. In the present study, plots of R_1 against concentration of eight GBCAs showed no consistent pattern and were not definitely linear or nonlinear (Fig. 5). Different serum total protein and albumin in each of experimental material setups could be the reason[20, 21]. Another reason for discrepancies can be attributed to inaccuracies of concentration determination and /or species-dependent influences of the blood plasma samples. One potential limitation of the current study is that blood oxygenation may influence blood T_1 values. However, conflicting conclusions regarding the influence of blood oxygen content have been reached in previous studies[15-19]. In Lin et al[16], rat blood T_1 values were found to be independent of oxygenation. The study of Rane et al[15] demonstrated that T_1 values of human blood at 7T differed depending on whether arterial or venous blood was examined. Other limitations include the fact that citrate was present in the blood in the present study, as discussed above in the methods section. However, a preliminary experiment demonstrated no differences in relaxivity values on this basis, thus justifying this approach. Also, different sources of blood were utilized, a fact that was accounted for by controlling for hematocrit fractions and ensuring that

serum total protein and albumin were similar. In addition, 3T measurements were obtained on samples of both the blood sources to further ensure standardization. The relaxivity values acquired at 3T from both sites/samples were similar. Lower Gd concentration than stated on the label may also have affected our results, however the relaxivity calculations reflect the formulation of the agents as commercially supplied (Table 2).

The current study provides T_1 relaxivity measurements, obtained from human whole blood, comparing the eight gadolinium chelates commonly employed world-wide as intravenous contrast media for magnetic resonance, at current relevant field strengths (1.5, 3, and 7T) and physiologic temperature. Statistically significant differences are noted both between agents, and between field strengths. The results from the present study should serve as a reasonable and reliable reference for further clinical utilization.

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Figures legends

Figure 1 Custom-built holder and phantom

All the tubes are placed parallel to each other in a custom-design acrylic holder, using plastic screws to sandwich the tubes and hold them in place. The holder was placed in the center of phantom with a removable cap. There was one hole in the center in which to place a temperature probe (*). The holder is shown in longitudinal (A) and transverse (B) views.

Figure 2 Analysis of data

(A) Regions of interests (ROIs) were automatically placed by the MATLAB program, signified by white rings (@3T, TI=60, zoomed in the right corner). Pixels inside the rings were measured. The temperature probe is denoted by the arrow.

(B) Mean SI values were utilized to plot T_1 relaxation curves. This particular example shows curves for Gd-EOB-DTPA at a concentration of 0.25 mM and 4mM at 3T.

(C) Plotting R_1 against concentrations to calculate the r_1 (at 3T for Gd-EOB-DTPA).

Figure 3 Graph illustrating SIs versus TIs of two protocols utilized to measure r_1 relaxivity at 1.5T and 3T. This example is of Gd-DOTA evaluated at a concentration of 0.0625mmol·L^{-1} at 1.5T, with protocol 1 and 2 (A), and at 3T with protocol 1 and 2 (B).

Figure 4 Calculation of human whole blood R_1 relaxation rate constant as performed using protocol 1 (A) and 2 (B) at 1.5T, as well as protocol 1 and 2 at 3T. Four control samples were included in the calculation.The calculated values were linearly dependent on B_0(C).

Figure 5 Illustrated is R_1 plotted against concentration for the 8 GBCAs at 1.5T, 3T and 7T.

Figure 6 An overview of the eight agents and their respective r1 relaxivities at 1.5, 3, and 7T is presented, in a graphical format.

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