Analytical Interference in Serum Iron Determination Reveals Iron Versus Gadolinium Transmetallation With Linear Gadolinium-Based Contrast Agents

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Objectives: The purposes of this study were to evaluate the risk for analytical interference with gadolinium-based contrast agents (GBCAs) for the colorimetric measurement of serum iron (Fe^{3+}) and to investigate the mechanisms involved.

Materials and Methods: Rat serum was spiked with several concentrations of all molecular categories of GBCAs, ligands, or "free" soluble gadolinium (Gd^{3+}). Serum iron concentration was determined by 2 different colorimetric methods at pH 4.0 (with a Vitros DT60 analyzer or a Cobas Integra 400 analyzer). Secondly, the cause of interference was investigated by (*a*) adding free soluble Gd^{3+} or Mn^{2+} to serum in the presence of gadobenic acid or gadodiamide and (*b*) electrospray ionization mass spectrometry.

Results: Spurious decrease in serum Fe^{3+} concentration was observed with all linear GBCAs (only with the Vitros DT60 technique occurring at pH 4.0) but not with macrocyclic GBCAs or with free soluble Gd^{3+}. Spurious hyposideremia was also observed with the free ligands present in the pharmaceutical solutions of the linear GBCAs gadopentetic acid and gadodiamide (ie, diethylene triamine pentaacetic acid and calcium-diethylene triamine pentaacetic acid bismethylamide, respectively), suggesting the formation of Fe-ligand chelate.

Gadobenic acid-induced interference was blocked in a concentration-dependent fashion by adding a free soluble Gd^{3+} salt. Conversely, Mn^{2+}, which has a lower affinity than Gd^{3+} and Fe^{3+} for the ligand of gadobenic acid (ie, benzyloxypropionic diethylenetriamine tetraacetic acid), was less effective (interference was only partially blocked), suggesting an Fe^{3+} versus Gd^{3+} transmetallation phenomenon at pH 4.0. Similar results were observed with gadodiamide. Mass spectrometry detected the formation of Fe-ligand with all linear GBCAs tested in the presence of Fe^{3+} and the disappearance of Fe-ligand after the addition of free soluble Gd^{3+}. No Fe-ligand chelate was found in the case of the macrocyclic GBCA gadoteric acid.

Conclusions: Macrocyclic GBCAs induced no interference with colorimetric methods for iron determination, whereas negative interference was observed with linear GBCAs using a Vitros DT60 analyzer. This interference of linear GBCAs seems to be caused by the excess of ligand and/or an Fe^{3+} versus Gd^{3+} transmetallation phenomenon.

Key Words: gadolinium-based contrast agents, transmetallation, interference, iron, mass spectrometry

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Gadolinium-based contrast agents (GBCAs) are widely used in magnetic resonance imaging (MRI) because of their unique capacity to enhance T1-weighted images by increasing the longitudinal relaxation rate of extracellular fluid protons ($1/T_1$).[1]

Some linear GBCAs have been reported to interfere with colorimetric assays for the determination of serum analytes[2] such as total calcium,[3–5] creatinine,[6] angiotensin-converting enzyme, zinc, magnesium, and iron.[7] The spurious decrease or increase in these analytes could lead to potential risks for the patient, such as wrong diagnosis or treatment.[8]

Interference with colorimetric assays can occur when, during or after GBCA dissociation, "unbound" Gd^{3+} binds to the dye or when the "free" ligand of GBCA associates with the analyte being measured.[7] In the context of the most widely reported analytical interference associated with gadolinium (Gd), spurious hypocalcemia, Lin et al[3] investigated the mechanism involved when calcium levels were measured by using *o*-cresolphthalein complexone (OCP). In the presence of the linear GBCA gadodiamide and OCP, they observed a disappearance of gadodiamide as well as an appearance of the free ligand diethylene triamine pentaacetic acid bismethylamide (DTPA-BMA) and a new complex, Gd-OCP. The interference of gadodiamide with colorimetric determination of serum calcium is therefore related to dissociation of this GBCA.[3] Nevertheless, some marketed GBCAs, especially macrocyclic chelates, do not generate any interference with analytical methods. This effect is therefore likely related to differences in GBCA thermodynamic and kinetic stability profiles.

Macrocyclic GBCAs are kinetically more stable than linear GBCAs, and linear ionic chelates are thermodynamically more stable than linear nonionic GBCAs.[9]

Under physiological conditions, endogenous cations such as Fe^{3+}, Zn^{2+}, or Cu^{2+} can compete with Gd^{3+} for the ligand and can induce a transmetallation phenomenon (ie, a metal exchange reaction), defined as follows:

$$GdL^n + M^{m+} \leftrightarrows Gd^{3+} + ML^{n+(m-3)} \quad (1)$$

where M is metal and L is ligand.

The probability of transmetallation depends on the affinity of endogenous metal ions for the ligand and is therefore related to the thermodynamic stability of the metal ion chelate (eg, Fe-L, Ca-L). Furthermore, some anions, such as PO_4^{3-}, CO_3^{2-}, and OH^-, can also compete with the ligand of GBCA at physiological pH, inducing precipitation of insoluble Gd salts.[10–15]

In a previous study performed in renally impaired rats, a negative interference with serum iron determination concentrations (spurious decrease in serum iron concentrations) was suspected after intravenous administrations of calcium-diethylene triamine pentaacetic acid (Ca-DTPA), gadobenic acid, or gadodiamide.[16]

The present study was therefore designed to evaluate the risk for analytical interference with all structural categories of GBCAs for colorimetric determination of serum iron levels by currently used laboratory methods and to investigate the cause of this interference.

MATERIALS AND METHODS

Analytical Interference of GBCAs, Gd Acetate, and Ligands With Colorimetric Determination of Serum Iron

Products

Five commercially available GBCAs were tested: a linear and nonionic GBCA, gadodiamide (Gd-DTPA-BMA, Omniscan; GE

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Healthcare, Chalfont St Giles, United Kingdom); 2 linear and ionic GBCAs, gadopentetic acid (Gd-DTPA; Magnevist, Bayer Healthcare, Berlin, Germany) and gadobenic acid (gadolinium-benzyloxypropionic diethylenetriamine tetraacetic acid [Gd-BOPTA], MultiHance, Bracco, Milan, Italy); a macrocyclic and nonionic GBCA, gadobutrol (gadolinium-butrol- 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, Gadovist; Bayer Healthcare, Berlin, Germany); and a macrocyclic and ionic GBCA, gadoteric acid (gadolinium-,4,7,10-tetraazacyclododecane-N,N′,N′,N″-tetraacetic acid, Dotarem; Guerbet, Villepinte, France).

The commercial solution of gadodiamide contains 5% calcium-diethylene triamine pentaacetic acid bismethylamide (Ca-DTPA-BMA) (caldiamide), and the pharmaceutical solution of gadopentetic acid contains 0.2% free diethylene triamine pentaacetic acid (DTPA) ligand. Gadolinium acetate ("free" soluble Gd^{3+}) as well as the Ca-DTPA-BMA and DTPA ligands were also tested.

Preparation of Solutions

Test solutions were prepared by diluting the various products in pooled rat serum (from healthy Wistar rats) or Seronorm (lyophilized human serum; InGen, Chilly-Mazarin, France). The following concentrations were tested: 0.05, 0.1, 0.5, 1.25, 2.0, 3.0, and 5.0 mM of Gd from each GBCA in rat serum or 5 mM of Gd from each GBCA in Seronorm. The Ca-DTPA-BMA and DTPA concentration ranges tested were calculated on the basis of the percentage of ligand present in the commercial solutions of gadodiamide and gadopentetic acid (ie, 5% and 0.2%, respectively[9]).

Colorimetric Determination of Serum Iron Concentration

After a 1-hour incubation at room temperature, serum iron concentration was determined using 2 different colorimetric methods with a Vitros DT60 analyzer (Ortho-Clinical Diagnostic, Issy les Moulineaux, France) or a Cobas Integra 400 analyzer (Roche Diagnostics, Meylan, France).

In the Vitros DT60 method (dry colorimetric method), ferric ion (Fe^{3+}) is released from transferrin at acidic pH (pH 4.0). Secondly, ferric iron is reduced to the ferrous form (Fe^{2+}) by ascorbic acid. Finally, ferrous iron is bound to a dye (3-pyridine sulfonamide reagent) and forms a final colored complex. Reflectance from the slide is read at 630 nm.

The Cobas Integra 400 method is a wet-chemical colorimetric method. Ferric iron (Fe^{3+}) is unbound from transferrin by guanidine hydrochloride (at pH 4.5). Ferric ion is subsequently reduced to ferrous iron by ascorbate and hydroxylamine. Bivalent iron ions form a colored complex with ferrozine, detected at 552 nm.

Determinations of iron serum levels were performed in duplicate, and all experiments were carried out twice. The percentage recovery of iron concentration values in the presence of test products was calculated in comparison with those measured in the absence of these compounds (percentage recovery).

No correction was made for dilution with the test solution because the error induced was negligible (<2%).

Mechanism of Interference

Colorimetric Determination of Serum Iron Levels by the Vitros Analyzer Technique

Linear GBCA

Gadolinium acetate (0.05–5.0 mM) or Mn chloride (1.25 and 5.0 mM) (Alfa Aesar, Schiltigheim, France) was added to pooled rat serum in the presence of either gadobenic acid (1.25 mM) or gadodiamide (2.0 mM). After a 1-hour incubation period, serum iron concentration was determined with the Vitros DT60 analyzer.

Role of Excess Ligand

Gadolinium acetate (2.0 mM) or Mn chloride (2.0, 2.5, 5.0, and 10.0 mM) was added to pooled rat serum in the presence of 2.0 mM of Ca-DTPA-BMA. After a 1-hour incubation, serum Fe^{3+} concentration was determined with the Vitros DT60 analyzer technique.

Mass Spectrometry

Mass spectrometry was used to investigate the possibility of Fe^{3+} versus Gd^{3+} transmetallation, after mixing gadobenic acid (1.25 mM), gadopentetic acid (2.0 mM), gadodiamide (2.0 mM), or gadoteric acid (2.0 mM) with iron chloride.

Study was performed by electrospray ionization mass spectrometry (ESI-MS) analysis using a quadrupole time-of-flight micromass spectrometer (Micromass, Manchester, United Kingdom) equipped with an electrospray ionization source and MassLynx version 4.0 software.

Preliminary tests, performed by infusion with commercial gadobenic acid (1.25 mM) solution, showed that an Fe^{3+} (iron chloride) concentration of 400 μM was necessary to obtain a specific response for iron chelate.

The effects of the addition of Gd acetate (5.0 mM) or Mn chloride (5.0 mM) to the test solution consisting of iron chloride and commercial aqueous solutions of GBCA were also tested.

The pH of the test solution was adjusted to pH 4.0 with formic acid and allowed to stand for 1 hour at room temperature before the ESI-MS analysis to mimic the Vitros DT60 analyzer technique conditions.

Because interference with ionization of compounds of interest, such as Gd chelate and Fe chelate, was observed in the presence of Gd acetate, all solutions were analyzed by high-performance liquid chromatography (HPLC) before introduction in the ionization source.

Because a single chromatographic condition could not be easily obtained for all products, several HPLC conditions were defined as follows:

- Symmetry C18 column (50 × 2.1 mm, 3.5 μm) with a water + 0.05% formic acid / acetonitrile + 0.03% formic acid gradient, injection of 5 μL, flow rate of 1 mL/min, and column oven set at 50°C for gadobenic acid;
- Ascentis RP Amide column (0 × 4.6 mm 3 μm) with a water + 0.05% formic acid / acetonitrile + 0.03% formic acid gradient, injection of 5 μL, flow rate of 1 mL/min, and column oven set at 50°C for gadopentetic acid;
- Alltima HP Hilic C18 column (150 × 4.6 mm 3 μm) with a gradient (phase A = water/acetonitrile, 76/24 vol/vol, with 12.5 of mM HCO_2H and 12.5 mM of HCO_2NH_4; phase B = acetonitrile/water, 76/24 vol/vol, with 12.5 mM of HCO_2H and 12.5 mM of HCO_2NH_4), injection of 5 μL, flow rate of 0.6 mL/min, and column oven set at 30°C for gadodiamide and gadoteric acid.

Electrospray source parameters were set as follows: ionization in positive mode; capillary voltage, 2.8 kV; cone voltage, 10 V; source temperature, 130°C; source desolvation, 350°C; as well as nitrogen flow rate for cone and desolvation at 35 and 700 L/h, respectively. Signal was acquired over the mass range of 120 to 1000 m/z (1 scan per second) after calibration with a 0.1% H_3PO_4 (volume per volume) aqueous solution.

Data analysis was performed using MassLynx software. For each GBCAs, the same number of scans was combined to compare the response of the compounds with various solution compositions (GBCA alone, with Fe^{3+}, Fe^{3+} with Gd^{3+}, Fe^{3+} with Mn^{2+}). Zoom around compounds of interest ligand and Fe ligand is presented, along with the response for each monoisotopic ion (named intensity).

Statistical Analysis

Data are expressed as mean. The limits used to define the presence of significant interference for the serum iron determination were those specified in the Clinical Laboratory Improvement Amendments

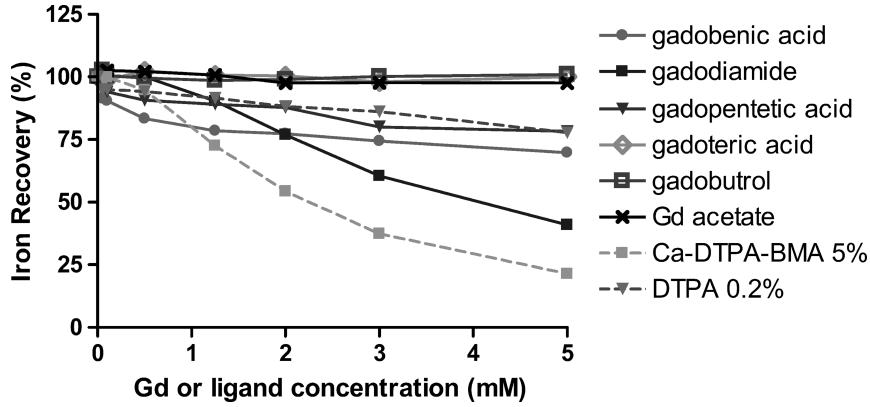

FIGURE 1. Effects of GBCAs and Gd acetate (0.05–5.0 mM in rat serum), Ca-DTPA-BMA (0.0025–0.25 mM), and DTPA (0.0001–0.01 mM) on the iron concentration determined by the colorimetric method with a Vitros DT60 analyzer (percentage recovery) (n = 2, in duplicate).

proficiency testing criteria for acceptable performance.[17] A serum iron test was considered correct within 20%.[17] Significant results were therefore defined as any interference with colorimetric determination of iron greater than 20%.

RESULTS

Analytical Interference of Commercial GBCAs in Colorimetric Determination of Serum Iron

The iron concentration of pooled rat serum (control values) was 35.8 ± 0.2 μmol/L (n = 4) using the Cobas Ingra 400 analyzer and 39.2 ± 1.0 μmol/L (n = 32) using the Vitros DT60 analyzer.

The measurement of rat serum iron using the guanidine/ferrozine method (Cobas Integra 400 analyzer) was not affected by the presence of the GBCAs tested (data not shown).

Rat serum iron levels determined by the Vitros DT60 analyzer did not differ from control values (serum alone) in the presence of Gd acetate (ie, free soluble Gd^{3+}), gadoteric acid, and gadobutrol at all concentrations tested (Fig. 1). However, a spurious decrease in serum iron levels was observed after addition of all linear GBCAs from 1.25 mM (gadobenic acid), 2 mM (gadodiamide), or 3 mM (gadopentetic acid) (interference exceeded 20%) (Fig. 1). This interference was concentration-dependent but not linear. The most pronounced interference was observed for gadodiamide (at 5 mM: iron recovery, 41%, ie, interference of 59%).

Human serum (Seronorm) iron concentration was 21.7 μmol/L (n = 2) using the Vitros DT60 analyzer. Analytical interference profiles obtained in Seronorm were similar to those observed in rat serum (Table 1). At 5-mM gadodiamide, recovery was 41% in rat serum and 38% in Seronorm (Table 1).

Analytical Interference of Gadodiamide, Gadopentetic Acid, and Their Respective Ligands in Colorimetric Determination of Serum Iron

A negative interference was observed between Ca-DTPA-BMA (tested at 5.0% of all test concentrations of the parent GBCA gadodiamide) and serum iron determination but slightly more pronounced with commercial gadodiamide (Fig. 1). In contrast, no interference was observed with Gd acetate.

A spurious concentration-dependent decrease in serum iron levels was also observed with the DTPA ligand (tested at 0.2% of the parent GBCA, gadopentetic acid), similar to that observed with commercial gadopentetic acid (Fig. 1).

Mechanism of Interference

Colorimetric Determination of Serum Iron Levels

Linear GBCA

Gadobenic acid (1.25 mM) produced a decrease in serum iron concentration of approximately 20% (Figs. 1, 2). The gadobenic acid-induced negative interference on serum iron determination was blocked by the addition of Gd acetate (free soluble Gd^{3+}) in a concentration-dependent fashion (interference was completely blocked from 1.25-mM soluble Gd^{3+} in the presence of 1.25-mM gadobenic acid). In contrast, the addition of soluble Mn^{2+} was less effective than that of soluble Gd^{3+} because inhibition of interference was not complete but partial with 5-mM Mn^{2+} (the serum iron concentration was decreased by approximately 8%) (Fig. 2).

Gadodiamide (2 mM) led to a spurious decrease in serum iron values (by approximately 23%; Fig. 1). The addition of free and soluble Gd^{3+} (5 mM) also completely blocked the analytical interference observed in the presence of 2-mM gadodiamide. As for gadobenic acid, soluble Mn^{2+} was less effective than soluble Gd^{3+} (data not shown).

Role of Excess Ligand

Gadodiamide (2 mM) produced a decrease in serum iron concentration of approximately 23% (Figs. 1, 3). At the same

TABLE 1. Effects of Several GBCAs, Ligands, and Gd Acetate on Rat or Seronorm Iron Determination Using a Vitros DT60 Analyzer (n = 2, in Duplicate)

Product (Concentration in Millimolar)	Iron Recovery (%)	
	Rat serum	Seronorm
Gadodiamide (5 mM)	41	38
Ca-DTPA-BMA (0.25 mM)	21	—
Gadopentetic acid (5 mM)	78	82
DTPA (0.01 mM)	78	78
Gadobenic acid (5 mM)	70	66
Gadoteric acid (5 mM)	100	100
Gadobutrol (5 mM)	101	101
Gd acetate (5 mM)	98	98

Ca-DTPA-BMA indicates calcium-diethylene triamine pentaacetic acid bismethylamide; DTPA, diethylene triamine pentaacetic acid; GBCA, gadolinium-based contrast agents; Gd, gadolinium.

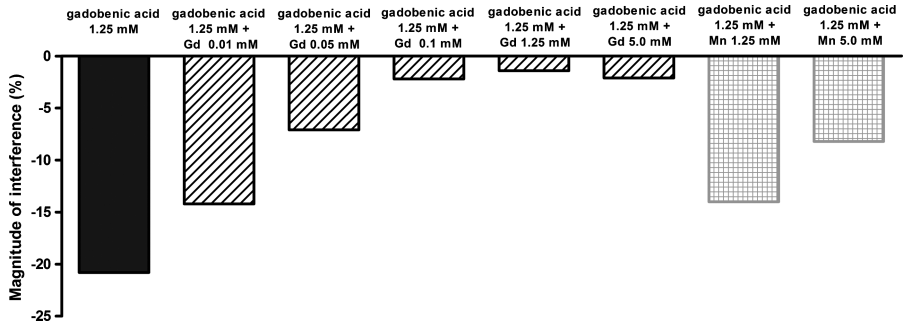

FIGURE 2. Effects of adding Gd acetate (0.01–5.0 mM in the rat serum) or Mn chloride (1.25 and 5.0 mM) in the presence of gadobenic acid (1.25 mM) on the iron concentration determined by the colorimetric method with a Vitros DT60 analyzer (magnitude of interference) (in duplicate).

concentration of Ca-DTPA-BMA (added ligand of gadodiamide pharmaceutical solution), the apparent serum iron values were below the lower limit of quantification (ie, 1.8 μM) and therefore not measurable (Fig. 3). However, the addition of soluble Gd^{3+} (2 mM) to Ca-DTPA-BMA (2 mM) partially blocked the Ca-DTPA-BMA–induced interference (decrease in iron levels of 9.6%) (Fig. 3). However, the addition of 2-mM soluble Mn^{2+} chloride was not sufficient to block Ca-DTPA-BMA-induced interference because at least 5-mM soluble Mn^{2+} had to be added to partially block Ca-DTPA-BMA-induced interference (Fig. 3).

Mass Spectrometry

In the pharmaceutical solution of gadodiamide, no free DTPA-BMA or Ca-DTPA-BMA was detected by mass spectrometry. After the addition of 400-μM Fe^{3+}, an increase in Fe-DTPA-BMA was observed (Fig. 4). The addition of 5-mM Gd^{3+} in the presence of 400-μM Fe^{3+} and gadodiamide resulted in an important decrease in Fe-DTPA-BMA response (by a factor of approximately 35), whereas no Mn-DTPA-BMA was detected after the addition of 5-mM Mn^{2+} (Fig. 4).

The presence of free ligand benzyloxypropionic diethylenetriamine tetraacetic acid (BOPTA) and Fe-BOPTA was detected in the gadobenic acid pharmaceutical solution alone. The mass spectrum of gadobenic acid (1.25 mM) (see Supplemental Figure 1, Supplemental Digital Content 1, which illustrates a zoom for BOPTA ligand and Fe-BOPTA detection in the ESI/MS spectra, http://links.lww.com/RLI/A160) showed an increase in Fe-BOPTA after the addition of 400-μM Fe^{3+} (intensity response × approximately 3), whereas no impact was observed on the BOPTA level. The addition of 5-mM soluble Gd^{3+} resulted in a decrease of the Fe-BOPTA signal (by a factor of approximately 2). Both Fe^{3+}-BOPTA ($C_{22}H_{28}FeN_3O_{11}$) and Mn^{2+}-BOPTA ($C_{22}H_{29}MnN_3O_{11}$) presented monoisotopic MH^+ ion at m/z 567 Th. However, no difference in the ratio of the 565Th/567Th peaks was observed between GBCA + Fe^{3+} in the presence or absence of Mn^{2+}. By taking into account the natural isotopic distribution of each metal (^{54}Fe, 5.8%; ^{56}Fe, 91.72%; ^{57}Fe, 2.2%; ^{58}Fe, 0.28%; and ^{55}Mn, 100%), our experimental data are suggesting the absence of Mn-BOPTA in the gadobenic acid solution after the addition of Mn chloride.

Similar findings for variations of the Fe-DTPA signal after the addition of 400-μM Fe^{3+} alone and then with 5-mM Gd^{3+} or 5-mM Mn^{2+} were observed for gadopentetic acid (see Supplemental Figure 2, Supplemental Digital Content 2, which illustrates a zoom for DTPA ligand and Fe-DTPA detection in the ESI/MS spectra, http://links.lww.com/RLI/A161).

No 1,4,7,10-tetraazacyclododecane-N,N′,N′,N″-tetraacetic acid ligand and no Fe-DOTA were detected in the pharmaceutical solution of gadoteric acid with or without the addition of 400-μM Fe^{3+} (see Supplemental Figure 3, Supplemental Digital Content 3, which illustrates the ESI/MS spectra for gadoteric acid in presence of 400-μM Fe^{3+}, http://links.lww.com/RLI/A162).

DISCUSSION

It has been previously reported that some linear GBCAs, used as MRI contrast agents, may interfere with colorimetric assays for the determination of several serum analytes.[2–7] Proctor et al[7] reported an interference with linear GBCAs gadodiamide, gadoversetamide, and gadopentetic acid for serum iron determination (Vitros 950 method) (0.00625–0.5 mM). The mechanism of this interference was unclear.

The present study investigated the possibility of an analytical interference of serum iron determination with the 4 molecular categories of GBCAs, using 2 widely used laboratory colorimetric methods, and also investigated the cause of this interference.

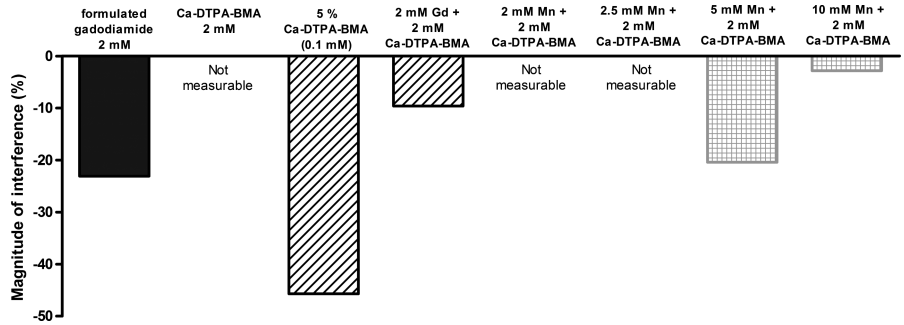

FIGURE 3. Effects of adding Gd acetate (2.0 mM) or Mn chloride (2.0, 2.5, 5.0, or 10.0 mM) in the presence of Ca-DTPA-BMA (2.0 mM) on the iron concentration determined by the colorimetric method with a Vitros DT60 analyzer (magnitude of interference) (in duplicate).

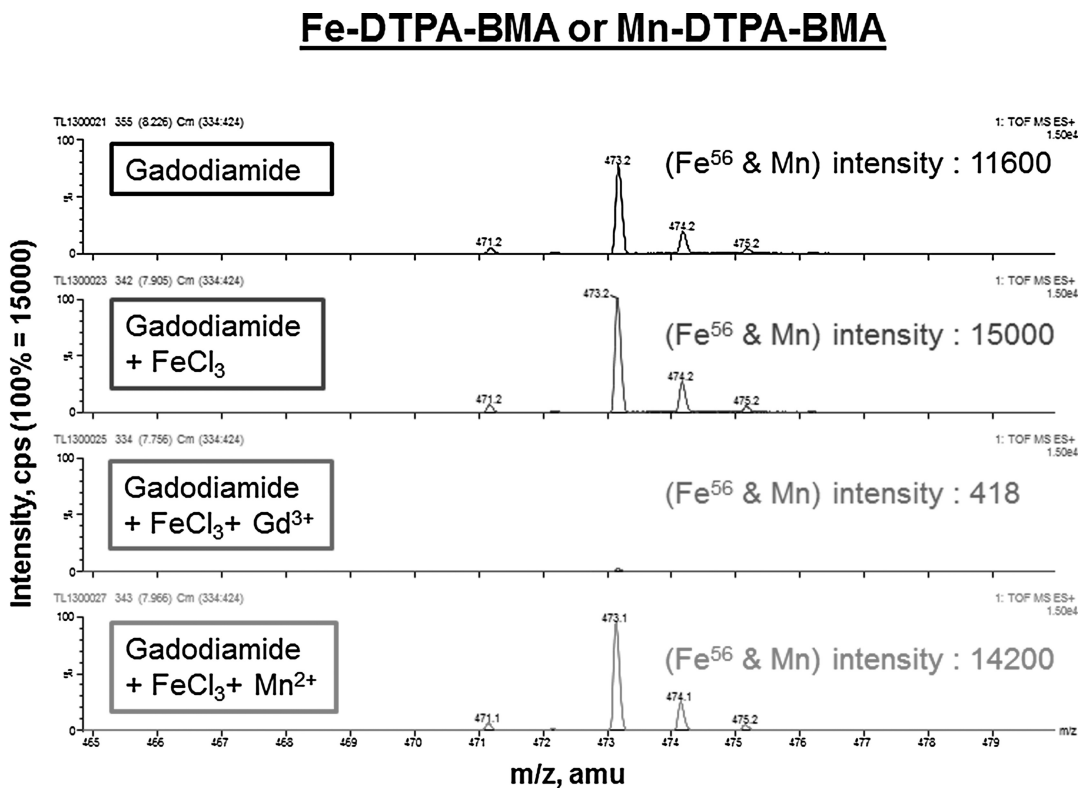

FIGURE 4. The ESI/MS spectra for gadodiamide solutions with a zoom for Fe-DTPA-BMA detection ($C_{16}H_{29}N_5O_8$, the molecular formula for DTPA-BMA ligand).

Most experiments were performed on rat serum because of the suspected analytical interference observed in a rat study.[16]

Under our study conditions, no interference was observed using the guanidine/ferrozine method (Cobas analyzer), regardless of the GBCA tested. Inversely, a negative analytical interference for the colorimetric assay of serum iron, using 3-pyridine sulfonamide as reagent (ie, the Vitros DT60 method), was observed with all linear GBCAs tested (gadobenic acid, gadopentetic acid, and gadodiamide), from a concentration of 1.25 mM depending on the chelate tested. In the subsequent mechanistic experiments, we selected the minimum concentration of GBCA for which there was an interference of 20% with colorimetric determination of iron as requested by the Clinical Laboratory Improvement Amendments guidelines.[17] In contrast, no interference was observed with macrocyclic GBCAs (gadoteric acid and gadobutrol) and free soluble Gd^{3+}, regardless of the concentration tested.

An interference was observed in pooled rat serum and in reconstituted human serum (Seronorm), despite the different iron concentrations observed in these 2 species when using the 3-pyridine sulfonamide–based method. These effects therefore seem to be independent of serum iron concentration and the species. However, the interference was proportional to the Gd concentration. The serum Gd concentration range tested was selected on the basis of human pharmacokinetic data. The pharmacokinetics of GBCAs is classically described by a 2-compartment model.[18] A maximum concentration of 2.5 mM could be expected in the intravascular compartment (for a circulating serum volume of 40 mL/kg) immediately after bolus intravenous administration of a GBCA clinical dose of 0.1 mmol/kg (for a body weight of 70 kg), assuming a strictly intravascular distribution.[3] In reality, nonspecific GBCAs are rapidly distributed in the extravascular space and their elimination half-life is approximately 90 minutes in healthy patients.[19] Therefore, shortly after intravenous injection (at the clinical dose of 0.1 mmol/kg), serum Gd concentrations would be approximately 0.5 to 1.0 mM.[20] An interference with iron determination can therefore be expected in clinical practice if blood is sampled shortly after the imaging procedure. Furthermore, GBCA excretion is prolonged in patients with renal impairment, especially in those patients with severe renal failure[21] and renal impairment who often receive iron supplements because of the presence of anemia. This specific population can therefore be considered to be at risk for this type of interference.

Spurious hyposideremia was also observed with both DTPA and Ca-DTPA-BMA, that is, the free ligands added in the pharmaceutical solutions of the linear GBCAs, gadopentetic acid, and gadodiamide, respectively, suggesting the formation of the Fe-ligand (Fe-L) chelate. The interference with these 2 linear GBCAs may therefore be explained, at least partly, by the presence of excess ligand in their pharmaceutical solution. An Fe^{3+} versus Ca^{2+} transmetallation phenomenon involving the added free Ca-DTPA-BMA ligand seems likely for the gadodiamide pharmaceutical solution when using the Vitros DT60 method. The thermodynamic stability of Ca-DTPA-BMA is lower than that of Fe-DTPA-BMA, thus favoring the formation of the latter chelate (Log K_{therm}: 7.17 versus 21.9, respectively; Table 2).[9,22]

The role of excess ligand was confirmed in an additional study. Free soluble Gd^{3+} partially blocked the Ca-DTPA-BMA ligand–induced interference because Gd^{3+} can bind to the DTPA-BMA ligand to form the Gd-DTPA-BMA chelate instead of Ca-DTPA-BMA (because of the higher Log K_{therm} for Gd-DTPA-BMA; Table 2).

The commercial solution of gadobenic acid is formulated without "further pharmaceutical excipients" (ie, no addition of free ligand in the pharmaceutical solution).[23] Therefore, the potential presence of an excess ligand does not exclude the possibility of a mechanism similar with gadobenic acid to what was actually observed with gadopentetic acid.

TABLE 2. Thermodynamic Stability Constant Log K_{therm} (ML) or Log K_{cond} (ML) of Various Chelates According to the Cation (Mn^{2+}, Ca^{2+}, Fe^{3+}, and Gd^{3+}) [9,22–25]

Ligand	Log K_{therm}GdL	Log K_{therm}CaL	Log K_{therm}FeL	Log K_{cond}GdL	Log K_{cond}FeL	Log K_{cond} MnL
DOTA	25.6	17.23	29.4	19.3	22.7	13.2
BOPTA	22.6	N/A	N/A	18.4	23.4	11.1
DTPA	22.1	10.75	28.0	17.7	23.4	11.0
DTPA-BMA	16.9	7.17	21.9	14.9	N/A	N/A

BOPTA indicates benzyloxypropionic diethylenetriamine tetraacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-N,N′,N′,N″-tetraacetic acid; DTPA, diethylene triamine pentaacetic acid; DTPA-BMA, diethylene triamine pentaacetic acid bismethylamide; K_{cond} MnL, conditional constant Mn-ligand; K_{cond}FeL, conditional constant Fe-ligand; K_{cond}GdL, conditional constant Gd-ligand; K_{therm}CaL, thermodynamic constant Ca-ligand; K_{therm}FeL, thermodynamic constant Fe-ligand; K_{therm}GdL, thermodynamic constant Gd-ligand; ML, metal-ligand; N/A, not available.

Because no interference was observed with the 2 macrocyclic GBCAs tested (ionic or nonionic) with colorimetric serum iron determination, an Fe^{3+} versus Gd^{3+} transmetallation mechanism (that is, the exchange between Gd^{3+} and Fe^{3+}, according to equation 1) was suggested to explain the interference observed with linear GBCAs. Two different methods were used to investigate this possibility.

Using the colorimetric method, the gadobenic acid–induced interference was blocked by the addition of free soluble Gd^{3+} in a dose-dependent fashion, consistent with the formation of the Gd-BOPTA chelate. This effect suggests a transmetallation phenomenon between Fe^{3+} and Gd^{3+} at pH 4.0. This phenomenon can be explained by the more favorable thermodynamic stability constant of the Fe-BOPTA chelate compared with that of Gd-BOPTA (Table 2; Log K_{cond}: 23.4 versus 18.4, respectively).[9,23] In contrast, the addition of soluble Mn^{2+} was less effective because complete inhibition of the gadobenic acid-induced Fe^{3+} interference was not observed at the high Mn^{2+} concentration of 5.0 mM. Indeed, the thermodynamic stability constant of Mn-BOPTA is lower than that of Gd-BOPTA (Log K_{cond}: 11.1 versus 18.4, respectively)[9,23] and that of the Fe-BOPTA (Log K_{cond}: 23.4). The affinities of Fe^{3+} and Gd^{3+} for the BOPTA ligand are therefore higher than that of Mn^{2+}. These data are consistent with this transmetallation hypothesis. Similar results were observed with the commercial solution of gadodiamide.

Mass spectrometry studies were performed to investigate the presence of Fe-L in aqueous samples containing GBCA with or without the addition of 400-μM iron at pH 4.0 (same acidic conditions as those of the colorimetric method). A 10-fold higher iron concentration compared with serum iron levels was used to potentiate the effects and to obtain a specific response for iron chelate. In the same way as in the "colorimetric study," soluble Gd^{3+} or Mn^{2+} was added to the test solution (ie, a mixture of commercial GBCA solutions with Fe^{3+}) to investigate the possibility of inhibition of Fe-L formation.

Fe-ligands (ie, Fe-BOPTA, Fe-DTPA-BMA, and Fe-DTPA) were detected with all linear GBCAs tested in the presence of Fe^{3+}, suggesting a transmetallation phenomenon between Fe^{3+} and Gd^{3+}. In contrast, no Fe-DOTA was observed in the gadoteric acid solution under similar conditions. This macrocyclic and ionic GBCA has higher kinetic stability and higher thermodynamic stability (Table 2).[9,22,24,25] The stronger affinity of this macrocyclic ligand for Gd^{3+} therefore prevents the Fe^{3+} versus Gd^{3+} transmetallation phenomenon at pH 4.0.[9,11]

The addition of soluble Gd^{3+} in the presence of Fe^{3+} and linear GBCAs resulted in a decrease in Fe-L response, suggesting the formation of Gd-L. However, the addition of soluble Mn^{2+} (lower affinity for the ligands tested; Table 2) had no major impact on the Fe-L response. These results are in agreement with our colorimetric findings.

In summary, in the presence of iron (endogenous iron ions in serum or addition of Fe^{3+} to the aqueous solution), linear GBCA can dissociate, a reaction probably catalyzed by acidic conditions and likely related to the intrinsically weaker stabilities of linear GBCAs compared with those of macrocyclic GBCAs, such as gadoteric acid.[20] The free linear ligand resulting from this reaction or present in the pharmaceutical solution may therefore bind Fe^{3+} (because of the high affinity of this metal for the ligand) by a transmetallation phenomenon. This metal exchange would induce the formation of a new chelate, Fe-L, and lead to a decrease in the measurable iron levels (determined by the colorimetric method), thus making serum iron "unavailable" for the colorimetric reaction with the 3-pyridine sulfonamide reagent. In contrast, using another technique (the guanidine/ferrozine method by Cobas analyzer), no interference was observed. These 2 colorimetric assays are performed under acidic conditions, but the Vitros DT60 technique only uses ascorbic acid as reducing agent, whereas the Cobas method uses both hydroxylamine and ascorbate. Our findings are not completely consistent with those previously reported by Proctor et al.[7] In our study, all linear GBCAs produced negative interference. However, in the study of Proctor et al,[7] gadopentetic acid also induced negative interference, whereas gadodiamide induced positive interference for the Vitros 950 method. These authors used also 2 analyzers using ferrozine as dye, but interference was only observed with the Synchron LX-20 analyzer and not with the Modular P analyzer. Results observed with the Modular P analyzer are consistent with our results.

Recently, the possibility of Fe^{3+} versus Gd^{3+} transmetallation with GBCAs (gadopentetic acid or gadoteric acid) and with endogenous plasma iron, citrate iron, or parenteral iron supplements (as administered to patients with renal impairment) was also investigated using an analytical method (HPLC and ESI-MS).[26] The time-dependent presence of Fe-DTPA was detected in plasma samples containing gadopentetic acid and iron citrate. Transmetallation of gadopentetic acid (Gd-DTPA) with either endogenous iron ions or parenteral iron supplements could not be proven under these experimental conditions, whereas no transmetallation with gadoteric acid was observed in any of the samples.[26] These results concerning gadoteric acid are in agreement with our findings.

CONCLUSIONS

Spurious hyposideremia was observed in the presence of all linear GBCAs tested but not with gadoteric acid, a macrocyclic GBCA, and free soluble Gd^{3+} when using the Vitros DT60 colorimetric assay. This interference of linear GBCAs with serum Fe^{3+} determination seems to be caused by the excess of ligand and/or an Fe^{3+} versus Gd^{3+} transmetallation phenomenon, probably related to the weaker stability of these GBCAs. Our data might be clinically relevant when serum iron is determined shortly after a contrast-enhanced MRI procedure or in patients with renal impairment because this interference may lead to unnecessary treatment and, therefore, iron overdose. In patients at risk for or in patients recently receiving linear GBCA administration, caution should be exercised when using this type of

colorimetric assay for serum iron determination and another colorimetric method should preferably be used.

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