Model for Predicting the Impact of Gadolinium on Plasma Calcium Measured by the o-Cresolphthalein Method

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Background: Gadolinium formulations, which are administered as contrast agents in magnetic resonance imaging examinations, interfere with colorimetric serum calcium determinations.

Methods: We performed an in vitro study to determine the extent to which three gadolinium formulations—gadodiamide (Omniscan), gadopentetate dimeglumine (Magnevist), and gadoversetamide (OptiMARK)—affect measurements by two methods that use o-cresolphthalein (Dade Behring, Inc. and Roche Diagnostics) and one that uses arsenazo dye (Equal Diagnostics). We also compared values from the o-cresolphthalein methods for 116 samples from patients administered gadodiamide.

Results: Magnevist did not affect any of the methods evaluated, whereas Omniscan and OptiMARK were identical in their effects. For the Dade method, the differences from the control sample were ≤4.0 and 7.0 mg/L at 0.25 and 0.5 mmol/L gadolinium, respectively. For the Roche method, the differences were 19, 9.0, and 5.0 mg/L at 0.5, 0.25, and 0.125 mmol/L gadolinium, respectively. Falsely increased calcium values were seen when samples were measured by the arsenazo-based method: differences were 6.0 and 3.0 mg/L at 1.0 and 0.5 mmol/L gadolinium. Using patient data collected at our institution, we were able to generate a model for predicting, from a patient’s glomerular filtration rate and the time elapsed since administration, the impact of Omniscan on calcium measurements by the o-cresolphthalein method from Roche Diagnostics.

Conclusions: The predictive model can be used to calculate, in patients who have received gadodiamide, the minimum length of time to wait before blood collection to avoid pseudohypocalcemia when the Roche o-cresolphthalein method is used.

Gadolinium, a lanthanide ion, is routinely administered intravenously as a contrast agent in magnetic resonance imaging (MRI)3 examinations. Characteristics of gadolinium that make it favorable as a contrast agent include its high paramagnetism and its exceptionally long electronic relaxation time. However, because of its interaction with calcium-dependent biological systems and calcium channels (1, 2) and its tendency to precipitate above pH 6, which lead to trapping in the liver and other phagocytic tissues, chelation of this ion by appropriate polyaminopolycarboxylic ligands is necessary for its clinical use. Gadolinium chelates have a favorable safety profile: they are associated with no clinical incidence of nephrotoxicity (3–5) and with an extremely low incidence of allergic reactions (6, 7). Currently, there are four gadolinium-based contrast agents available commercially in the US: gadodiamide (Omniscan; Amersham Health), gadopentetate dimeglumine (Magnevist; Schering), gadoversetamide (OptiMARK; Mallinckrodt Medical), and gado- teridol (Prohance; Bracco).

In April 2002, the makers of Omniscan, which is the contrast agent most often used at our institution, issued a change in product labeling to state that Omniscan interferes with colorimetric serum calcium determinations, producing falsely low calcium measurements. Roche Diagnostics also issued a user bulletin at this time alerting laboratories to the effect of this agent in their o-cresol-

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3 Nonstandard abbreviations: MRI, magnetic resonance imaging; OCP, o-cresolphthalein; and GFR, glomerular filtration rate.
phthalein (OCP)-based calcium method. This problem has been documented for gadodiamide in colorimetric calcium methods based on the calcium binding dyes OCP (8–11) and Arsenazo III (9). The mechanism of the interference was demonstrated by performing ultraviolet spectrometry and electro spray ionization mass spectrometry on mixtures of OCP and various gadolinium chelate formulations (8). This study demonstrated that the interference is attributable to complete displacement of the gadolinium$^{3+}$ ion from gadodiamide by OCP, followed by the formation of a new gadolinium complex: 2GD–2OCP. The formation of this complex could also explain the change in ultraviolet absorbance of OCP (which is responsible for the decreased calcium measurements) observed in the presence of gadodiamide. Normann et al. (9) demonstrated that gadolinium chelates do not affect calcium measurements that use ion-selective electrodes or inductively coupled plasma–atomic emission spectroscopy.

Before the manufacturers’ notifications, we had noticed at least three patients with unexpectedly low calcium measurements after contrast-enhanced MRI studies and that these unexplained periods of hypocalcemia persisted for up to 2 days. Interestingly, this was far longer than would be predicted by the 78-min half-life of gadodiamide. Spuriously low calcium measurements have been reported in patients with renal insufficiency for up to 4.5 days after gadodiamide administration (10). We therefore aimed to determine three things regarding this phenomenon: (a) the effect of different gadolinium preparations on calcium values; (b) the magnitude of this effect in three different calcium measurement methods; and (c) to what extent impaired glomerular filtration rate (GFR) would lengthen the time that these agents would adversely affect calcium values.

We examined the in vitro effect of three different gadolinium formulations on calcium measurements obtained by two OCP-based calcium methods as well as their effect on an arsenazo-based method. We then examined the in vivo effect of gadodiamide administration on calcium measurements in 116 samples from 99 patients, concentrating on the intermediate post administration period (1–24 h after administration), which had not been evaluated previously. The goal was to generate a model for predicting, from a patient’s GFR and the time elapsed since administration of gadodiamine, the impact of this agent on plasma calcium values obtained by a very commonly used method from Roche Diagnostics. Such a model could be useful in alerting clinicians to the possibility of spuriously low plasma calcium values and preventing unnecessary workup as a result of these laboratory values.

**Materials and Methods**

**IN VITRO INTERFERENCE STUDIES**

Plasma samples with calcium values within the reference interval obtained from Barnes-Jewish Hospital Laboratory (St. Louis, MO) were pooled for this experiment. The gadolinium-based contrast agents gadodiamide (Omniscan; Amersham Health), gadopentetate dimeglumine (Magnevist; Schering), and gadoversetamid (OptiMARK; Mallinckrodt Medical) were obtained from the respective manufacturers. We added 20, 10, 5, 2.5, 1.25, and 0.63 μL of each gadolinium formulation to 5 mL of pooled plasma for final concentrations of 2, 1, 0.5, 0.25, 0.125, and 0.063 mmol/L, respectively. Equal volumes of doubly distilled H$_2$O were added to pooled plasma for the controls. Plasma calcium values were measured on the Dade Dimension RxL (Dade Behring, Inc.) and Hitachi 747 (Roche Diagnostics.) with the respective manufacturer’s OCP method. The arsenazo method was from Equal Diagnostics and was performed on a Hitachi 747 analyzer.

**PATIENTS**

The laboratory was notified by the radiology department of any patient receiving gadolinium contrast medium between August 16, 2002, and October 28, 2002, and whether a calcium value had been requested for that patient. Calcium concentrations were determined by two different OCP methods: one from Roche Diagnostics performed on the Hitachi 747 analyzer and one performed on the Dade Behring Dimension analyzer. A total of 116 samples from 99 patients were used in this study. This study was approved by the Human Studies Committee of Washington University.

**ESTIMATED GFR**

GFR was estimated by the previously described MDRD equation (12):

$$\text{Estimated GFR} = 186 \times (\text{Scr})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$$

where Scr is serum creatinine.

**STATISTICAL ANALYSIS**

The difference between calcium values measured by the Roche and Dade methods was modeled as a function of two predictor variables: estimated GFR and time since administration of contrast medium. The best fitting model was derived by use of fractional polynomials (13). This approach involves selecting the best transformation for each predictor variable from a set of fractional polynomial powers. The transformations that were evaluated for each predictor variable included $1/x^2$, $1/x$, $1/\sqrt{x}$, $\ln(x)$, $\sqrt{x}$, $x^2$, and $x^3$. ROC curves and the area under the ROC curve are calculated nonparametrically. Exact 95% confidence intervals are reported for sensitivities and specificities.

**Results**

Given a standard dose of 0.1 mmol/kg and a plasma volume of 40 mL/kg, and assuming a strictly intravascular distribution (which is never achieved in practice), the theoretical maximum gadolinium concentration in plasma
would be 2.5 mmol/L. The calcium measurements in aliquots of pooled plasma with different concentrations of gadolinium are shown in Fig. 1. Whereas Magnevist (gadopentate dimeglumine) did not affect calcium measurements by any of these methods (data not shown), Omniscan (gadodiamide) and OptiMARK (gadoversetamide) had a profound effect on the Roche OCP-based method and a lesser, but still significant, effect on the Dade OCP method. At gadolinium concentrations of 0.25 and 0.5 mmol/L, the differences in the measured calcium values from control samples were ≤4.0 and 7.0 mg/L by the Dade method. In contrast, with the Roche method, the differences were 19.0, 9.0, and 5.0 mg/L at gadolinium concentrations of 0.5, 0.25, and 0.125 mmol/L, respectively. Samples with Omniscan (gadodiamide) or OptiMARK (gadoversetamide) also gave a falsely increased calcium value when measured by the arsenazo-based method: differences of 6.0 and 3.0 mg/L at gadolinium concentrations of 1.0 and 0.5 mmol/L, respectively. For each calcium method, there was little difference in the interfering effects of Omniscan and OptiMARK.

Of the 116 samples in this study, 33 (28%) had calcium values that were more than 5.0 mg/L lower in the Roche method than in the Dade method. Because the Dade RXL also suffers from a small amount of negative interference, the actual number of significantly falsely decreased calcium values is most likely slightly higher than this.

In patients receiving gadodiamide, we wished to determine the impact of GFR and time since administration on the observed differences in calcium values between the Roche and Dade OCP methods. As shown in Fig. 2, the magnitude of the difference in calcium measurements was inversely related in a nonlinear fashion to both the magnitude of the estimated GFR and the length of time since administration of contrast medium. The best fractional polynomial predictor of the difference in calcium measurement is shown in Fig. 3. The curved surface gives the predicted difference in calcium measurement (Ca Diff) as a function of estimated GFR and elapsed time since administration of contrast medium (Hr). The equation that defines the predicted difference is:

\[
Ca\ Diff = 17.909 - 4.927\ln(GFR) + \frac{21.316}{\sqrt{Hr}}
\]

Rather than predicting the actual magnitude of the difference between calcium measurements, we were more interested in deriving a prediction rule that would have good sensitivity and specificity for predicting differences that exceed 5.0 mg/L. Any curved line defined along the surface of the fractional polynomial predictor in Fig. 3 that
is a constant height above the “floor” of Fig. 3 can be used as a decision boundary for predicting “discrepant” calcium results. Three example curves, corresponding to differences of 6.15 (Fig. 3A), 4.6 (Fig. 3B), and 2.8 mg/L (Fig. 3C), are shown. Fig. 4 shows these same three decision boundaries in a two-dimensional plot of elapsed time since administration of contrast medium vs estimated GFR.

For each decision boundary, the sensitivity and specificity of predicting discrepant calcium measurements can be calculated. A ROC curve can be generated by calculating the sensitivity and specificity for a range of decision boundaries. This is shown in Fig. 5. The area under the ROC curve for the fractional polynomial predictor is 0.9215. Fig. 5 also shows the sensitivities and specificities for the three decision boundaries labeled A, B, and C in Figs. 3 and 4. The equation for predicting discrepant calcium results, using the decision boundary labeled A is:

\[ \text{GFR} < e^{(2.3867 + 4.3265/\sqrt{Hr})} \]

or

\[ Hr < \frac{4.5438}{[0.4927 \ln(\text{GFR}) - 1.1759]^2} \]

Thus, if the estimated GFR is less than the value obtained by the first equation based on the number of hours since administration of contrast medium or if the number of hours since administration of contrast medium is less than the value obtained by the second equation based on the estimated GFR, then the difference in calcium measurement would be predicted to exceed 5.0 mg/L. The sensitivity for predicting discrepant results using this decision rule is 91% (95% confidence interval,
76–98%), and the specificity is 89% (80–95%). Table 1 gives the minimum elapsed time since administration of contrast medium corresponding to different estimated GFR values to obtain a calcium measurement by the Roche method that is less 5.0 mg/L different from the Dade method.

**Discussion**

This study was initiated after samples from three patients with unexpectedly low calcium values from the Roche method on the Hitachi 747 showed discrepant results when measured by a different method (Dade Dimension RXL; Table 2). Further investigation revealed that all of the patients had received injections of gadodiamide within the last 2 days.

The results of the in vitro evaluation of gadolinium interference demonstrate that the Dade method is less susceptible to this negative interference than the Roche method. Although the Dade method has not been evaluated before for this effect, the results for the Roche method agree with previous studies (8, 10) and the Roche customer bulletin. Because the two instruments both use an OCP-based colorimetric method for calcium measurement, the reason for this is not immediately obvious. The total displacement of gadodiamide from its ligand by OCP could be related to the weaker stability constant of gadodiamide compared with other gadolinium chelates. Gadodiamide and gadoversetamide have stability constants of log \( K_{eq} = 16.9 \) and 16.6, respectively, whereas that of gadopentate dimeglumine is 22.1. The stability constant of gadoteridol, which is another chelate that does not affect the OCP method, is 25.8 (10). Because the instruments used in this study measure absorbance at slightly different pH values (pH 10.4 for the Roche and pH 9.6 for the Dade) and wavelengths (600 nm for the Roche, and 577 and 540 nm for the Dade), we think that one of these factors may be responsible for the fact that less interference is seen with the Dade method.

Because the Dade RXL also gives slightly decreased values, the cutoff of 6.15 mg/L would correspond to a false decrease of ~8–10 mg/L on the Hitachi 747. Although an error of 5.0 mg/L may be clinically significant, an error of 10 mg/L is the CLIA-defined acceptable error for calcium determinations. Our results for the arsenazo-based method examined here conflict with a study that reported negative interference when a different arsenazo method (Arsenazo III from Kodak Ectachem) was used (9).

Approximately 20,000 MRI examinations are performed annually at the Mallinckrodt Institute of Radiology of Barnes-Jewish Hospital. Of these, 50% are neurobrain and spine MRI, 30% are body MRI, and 20% are musculoskeletal MRI. The top indications for body MRI are liver, pancreas, angiography, kidney, and uterus imaging. Ninety-eight percent of body, 80% of neuro, and 20–30% of musculoskeletal MRI cases receive intravenous gadolinium chelate contrast medium. Thus, at least 14,000 patients/year receive gadolinium chelate infusions. We found that calcium determinations are ordered within 24 h of the imaging studies for 3.6% of these patients. In this study, we discovered that at least 28% of these cases had a clinically significant error when their plasma calcium was measured by the Roche OCP method. Prince et al. (10) reported that a decrease >20 mg/L in the plasma calcium measurement was noted in 42 (4%) of 1049 patients who had received gadodiamide infusions. All of these patients were in the hypocalcemic range, with 25 being in the critical range. However, none of these patients was noted to have symptoms characteristic of hypocalcemia. Thus, it is important to note that the decreased calcium values seen in these situations are the result of a phenomenon that occurs during measurement and do not reflect the actual plasma calcium concentrations of the patients.

We were able to generate a model to predict, from a patient’s GFR and the time elapsed since administration of gadodiamide, the impact of this agent on plasma calcium values measured by the Roche OCP method. This model can be used to calculate the minimum length of time after gadodiamide injection that is required to exclude pseudohypocalcemia in these patients when the

| Table 1. Recommended minimum waiting time since administration of contrast medium before collecting plasma sample for calcium measurement. |
|------------------|------------------|
| If estimated GFR is | Elapsed time should be ≥ |
| 20 | 50 h |
| 30 | 18 h |
| 40 | 11 h |
| 50 | 8 h |
| 60 | 6 h |
| 75 | 5 h |
| 90 | 4 h |
| 130 | 3 h |

Fig. 5. ROC curve (for predicting differences >5.0 mg/L) for decision boundaries used to predict discrepant calcium results.

A, 6.15 mg/L; B, 4.6 mg/L; C, 2.8 mg/L.
Roche OCP method is used. During this period, or when a patient’s renal function is unknown, other methods, such as ionized calcium measurement (9), could be used to measure plasma calcium. In patients with severely impaired renal function, the interference described above can occur up to 188 h after gadodiamide infusion (10). Therefore, it is difficult to specify a safe time period that would be applicable to all patients who had received gadodiamide if their renal function is unknown.

References


Table 2. Discrepant calcium measurements and gadodiamide injection dates for three patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gadolinium administration</th>
<th>Blood sample drawn</th>
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<th>RxL</th>
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