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Interference of gadolinium-based contrast agents on colorimetric calcium assays

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ABSTRACT

Objective: The aim of this study was to evaluate the potential interference of five gadolinium-based contrast agents (GBCAs), gadodiamide (Omniscan®), gadobenate dimeglumine (Multihance®), gadoxetate disodium (Primovist®), gadobutrol (Gadovist®), and gadoteridol (Prohance®), on three clinical laboratory widely used colorimetric calcium assays including the newly developed 5-nitro-5′methyl-l,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (NM-BAPTA) method.

Methods: Plasma was collected from healthy volunteers aged 23–52, and spiked with varying concentrations of the five GBCAs. Calcium determinations were performed in duplicates using the o-cresolphthalein complexone (OCP), arsenazo-III dye, and NM-BAPTA methods on the Roche Integra 400, Abbott Architect 16000, and Roche Modular P automated analyzers respectively.

Results: Gadobenate dimeglumine, gadobutrol, and gadoteridol did not interfere with any of the assays. There was a small positive bias (8%, $p < 0.01$) at a very high concentration (25 mmol/L) of gadoxetate disodium when calcium was assayed using the arsenazo-III method. Gadodiamide at a very high concentration (50 mmol/L) induced a significant positive bias (16%, $p < 0.01$) on calcium when measured using the NM-BAPTA method; however a much larger bias (90%, $p \ll 0.01$) was observed when calcium was measured using the arsenazo-III method. Significant interferences in calcium measurements using the OCP method began at gadodiamide concentrations as low as 0.5 mmol/L (−9%, $p < 0.01$). This negative bias was more pronounced at higher gadodiamide concentrations.

Conclusions: Of all 5 GBCAs tested, only gadodiamide showed significant interference on the OCP calcium assay at clinically relevant concentrations. The NM-BAPTA assay showed minimum interference with the five GBCAs and demonstrated equal or better performance than the OCP and the arsenazo-III methods in terms of interference with GBCAs.

Introduction

Gadolinium-based contrast agents (GBCAs) are routinely administered to enhance magnetic resonance imaging (MRI) by increasing lesion visibility, characterizing lesion perfusion, and highlighting vascular structures. Several extracellular chelates of gadolinium ion (Gd^{+++}) have been developed for clinical use. Based on molecular configurations, GBCAs are either linear or cyclic, and are available as ionic or non-ionic preparations [1]. In general, GBCAs are very safe and effective for diagnostic MRI applications [2], however it has been demonstrated that certain gadolinium containing MRI contrast agents such as gadodiamide (Omniscan®) and gadoversetamide (OptiMARK®) exhibit negative interference with some calcium assays [3–9]. Spurious life-threatening hypocalcemia cases have been reported in blood samples taken shortly after gadodiamide injections for MRI scans [5,6,8,9]. Interestingly, different inference patterns from GBCAs have been described on certain calcium colorimetric assays but not on the ion-selective electrode method, or inductively coupled plasma mass spectrometry (ICP-MS) methods [3,4,7,10]. In dose response studies, Löwe et al. [10,11] have demonstrated that gadodiamide and gadoversetamide had significant negative interference on the o-cresolphthalein complexone (OCP) method on Roche Cobas Integra and Mira analyzers at their 5 mmol/L level but positive biases on the arsenazo-III dye method on Ortho Vitros 950 and Abbott Architect analyzers at their 50 mmol/L level. Performing IV injections of therapeutic dosages of GBCAs to adult human healthy volunteers, Brown et al. [12] have shown that gadodiamide and gadoversetamide

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significantly decreased serum calcium values when measured by the OCP method from 5 to 60 min after administration, however calcium values by the arsenazo-III and ICP-MS methods were not affected.

A number of routine clinical methods exist to measure calcium concentrations in serum or plasma. According to the 2013 College of American Pathologists (CAP) Chemistry/Therapeutic Drug Monitoring survey (C-A), nearly half of the clinical laboratories that participated in the survey used an OCP colorimetric assay (2806 of 6076 laboratories), one third used an arsenazo-III dye method (2110 laboratories), one sixth used the ion selective electrode method (1084 laboratories) with the remainder using the 5-nitro-5′methyl-l,2-bis(2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (NM-BAPTA) method (54 laboratories) [13]. NM-BAPTA is a new calcium binding chelating agent and has advantages over the OCP method in that calcium sensitivity is not dependent upon a high pH of 10.7. A very high alkaline pH contributes to calcium assay performance variations because the reagent readily absorbs ambient carbon dioxide. The NM-BAPTA calcium method is also better than the arsenazo-III method because of the higher sensitivity and no environmental concern of arsenic contamination. Roche Diagnostics released the 2nd generation calcium assay in early 2013 using NM-BAPTA and made claims that this assay is free of interference from gadodiamide and gadoversetamide at therapeutic concentrations [14]. These claims have not been validated in literature nor has data for potential interference from other GBCAs been published. Because the measurement of serum/plasma calcium levels has an important impact on clinical practice, especially in reliably identifying patients with hypo- or hypercalcemia, it is important to characterize assay interference with these contrast agents [8].

Materials and methods

Gadolinium-based contrast agents

Five GBCAs available on the Canadian market were examined including gadodiamide (Omniscan®, GE Healthercare, Mississauga, Canada), gadobenate dimeglumine (Multihance®, Bracco Imaging, Montreal, Canada), gadoxetate disodium (Primovist®, Bayer Healthcare, Toronto, Canada), gadobutrol (Gadovist®, Bayer Healthcare, Toronto, Canada), and gadoteridol (Prohance®, Bracco Imaging, Montreal, Canada). Depending on the chelating molecule, each contrast agent has a unique molecular structure [1]. The target blood concentrations of GBCAs were estimated according to Löwe et al. [10,11] which was consistent with the gadolinium *in vivo* pharmacokinetic data from Brown et al. [12]. Briefly, the target blood concentration of gadoxetate disodium at the recommended dose of 0.025 mg/kg in a 70-kg patient with a 5-L blood pool is 0.35 mmol/L. The target blood concentration of gadodiamide, gadobenate dimeglumine, gadobutrol, and gadoteridol at the recommended dose of 0.1 mmol/kg in a 70-kg patient with a 5-L blood pool is 1.4 mmol/L. In certain applications, the dosages of gadobutrol and gadoteridol can be raised by a factor of 3, giving approximately 4.2 mmol/L. Dosage data for these five GBCAs are summarized in Table 1 with their respective estimated blood concentrations.

Gadobutrol was diluted 1:1 with type I water to make a 0.5 mol/L stock solution. Gadodiamide, gadobenate dimeglumine, and gadoteridol were supplied at 0.5 mol/L and gadoxetate disodium was supplied at 0.25 mol/L from the manufacturers (Table 1). Serial dilutions were made from the contrast agents with type I water to achieve a total of 5 stock solutions for each GBCA (500, 50, 5, 0.5, and 0.05 mmol/L for gadodiamide, gadobenate, gadobutrol, and gadoteridol; 250, 25, 2.5, 0.25 and 0.025 mmol/L for gadoxetate disodium).

Blood samples

Eighteen milliliter of blood was collected from 2 healthy male and 3 healthy female volunteers aged 23–52, into lithium heparin plasma separator tubes (PST™, Becton-Dickinson, Mississauga, Canada). Tubes were centrifuged at 1400 *g* for 10 min and plasma samples were obtained. Spiking of contrast agents was done by adding 20 μL of stock contrast solutions to 180 μL of plasma. As a dilution control (0 mmol/L GBCAs), 20 μL of type I water was added to 180 μL of plasma. An aliquot of 200 μL of undiluted patient plasma was used as a neat control. Final plasma concentrations of contrast agents for gadodiamide, gadobenate dimeglumine, gadobutrol, and gadoteridol were 50, 5, 0.5, 0.05, 0.005, and 0 mmol/L; for gadoxetate disodium were 25, 2.5, 0.25, 0.025, 0.0025 and 0 mmol/L. This study was approved by the Horizon Health Network Research Ethics Board and volunteer informed consent was obtained.

Calcium measurements

Samples were analyzed in duplicate by all three CAP survey-listed colorimetric methods [13]. The Roche Modular P platform (at the Dr. Everett Chalmers Regional Hospital) uses the 2nd generation assay with calcium binding NM-BAPTA. The calcium–NM-BAPTA complex then binds with EDTA to cause an absorbance change which is directly proportional to the calcium concentration and is measured photometrically. Samples were also analyzed on the Roche Cobas Integra 400 platform (at the Upper River Valley Hospital) using the OCP assay which utilizes the chromogen o-cresolphthalein to bind calcium and result in a purple-colored complex. The color intensity of the complex formed is directly proportional to the calcium concentration and is measured photometrically. The Abbot Architect 16000 (at the Moncton Hospital) uses arsenazo-III dye reacting with calcium in an acid solution to form a blue–purple complex. The color developed is measured at 660 nm and is proportional to the calcium concentration in the sample. To avoid between run imprecision, all specimens were measured within one analytical run.

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was carried out using the SPSS 20.0 software (IBM Corporation, Armonk, New York, USA) for one-way ANOVA. $p < 0.05$ was considered as statistically significant. Charts were created in the Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA),

Results

For all GBCA spiking experiments, there was an expected 10% drop in measured values of the 0 mmol/L GBCA samples diluted/spiked with

Table 1
Summary of tested gadolinium-based MRI contrast agents (GBCAs).

GBCA	Molecular structure Type	Packaging concentration	Recommended dosage	Estimated blood concentration (in a 70-kg person)
Gadodiamide (Omniscan®)	Non-ionic linear	0.5 mol/L	0.1 mmol/kg	1.4 mmol/L
Gadobenate dimeglumine (Multihance®)	Ionic linear	0.5 mol/L	0.1 mmol/kg	1.4 mmol/L
Gadoxetate disodium (Primovist®)	Ionic linear	0.25 mol/L	0.025 mmol/kg	0.35 mmol/L
Gadobutrol (Gadovist®)	Non-ionic cyclic	1.0 mol/L	0.1–0.3 mmol/kg	1.4–4.2 mmol/L
Gadoteridol (Prohance®)	Non-ionic cyclic	0.5 mol/L	0.1–0.3 mmol/kg	1.4–4.2 mmol/L