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IV Administered Gadodiamide Enters the Lumen of the Prostatic Glands: X-Ray Fluorescence Microscopy Examination of a Mouse Model

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Abstract

OBJECTIVE—Dynamic contrast-enhanced MRI (DCE-MRI) has become a standard component of multiparametric protocols for MRI examination of the prostate, and its use is incorporated into current guidelines for prostate MRI examination. Analysis of DCE-MRI data for the prostate is usually based on the distribution of gadolinium-based agents, such as gadodiamide, into two well-mixed compartments, and it assumes that gadodiamide does not enter into the glandular lumen. However, this assumption has not been directly tested. The purpose of this study was to use x-ray fluorescence microscopy (XFM) imaging in situ to measure the concentration of gadodiamide in the epithelia and lumens of the prostate of healthy mice after IV injection of the contrast agent.

MATERIALS AND METHODS—Six C57Bl6 male mice (age, 28 weeks) were sacrificed 10 minutes after IV injection of gadodiamide (0.13 mmol/kg), and three mice were sacrificed after saline injection. Prostate tissue samples obtained from each mouse were harvested and frozen; 7- μ m-thick slices were sectioned for XFM imaging, and adjacent 5- μ m-thick slices were sectioned for H and E staining. Elemental concentrations were determined from XFM images.

RESULTS—A mean ($\pm$ SD) baseline concentration of gadolinium of 0.01 ± 0.01 mM was determined from XFM measurements of prostatic tissue samples when no gadodiamide was administered, and it was used to determine the measurement error. When gadodiamide was added, the mean concentrations of gadolinium in the epithelia and lumens in 32 prostatic glands from six mice were 1.00 ± 0.13 and 0.36 ± 0.09 mM, respectively.

CONCLUSION—Our data suggest that IV administration of gadodiamide results in uptake of contrast agent by the glandular lumens of the mouse prostate. We were able to quantitatively determine gadodiamide distributions in mouse prostatic epithelia and lumens.

Keywords

dynamic contrast-enhanced MRI; gadodiamide distribution in prostatic tissues; mouse prostate; prostatic lumen; x-ray fluorescence microscopy

Dynamic contrast-enhanced MRI (DCE-MRI), which is commonly embraced as one of the main sequences comprising the multiparametric MRI paradigm for prostate imaging, is included in recently published guidelines [1]. A recent meta-analysis showed that DCE-MRI is promising for the detection of prostate cancer (PCa), with relatively high pooled specificity (87.9%) but poor sensitivity (55.3%), and with highly variable results between the included studies [2]. In addition, if it can provide reproducible results, DCE-MRI may have potential for assessing the response to androgen deprivation therapy, radiation therapy, and antiangiogenic and vascular targeting agents in PCa [3, 4].

Most recent literature on DCE-MRI of PCa utilizes quantitative analysis techniques that require pharmacokinetic models that are applied to changes in tissue signal over time to estimate in vivo gadodiamide concentrations [2, 5]. The two-compartment model is the most commonly used model for DCE-MRI analysis of PCa, and quantitative parameters such as K^{trans} (the transfer constant between plasma and extravascular extracellular volume) and k_{ep} (the contrast agent back flux rate constant) are derived from this model [6–8]. Although, on average, K^{trans} values are higher in PCa than in healthy prostatic tissue [9–13], the range of values for the healthy prostate and for PCa is very large within each study and is even larger between independent studies. In the literature, reported K^{trans} values are $0.1\text{--}1\text{ min}^{-1}$ for PCa [12, 13]. This variability suggests that there is a lack of standardization and significant random or systematic error, which limits clinical applications of DCE-MRI.

One source of systematic error may result from analyzing DCE-MRI data on the basis of the use of physiologic models that do not accurately describe the distribution of contrast media. The two-compartment model is based on the assumption of redistribution of gadolinium-based contrast agents, such as gadodiamide, into two compartments (intravascular and extravascular extracellular compartments) after injection of contrast agent [6]. In the prostate, it is always assumed (but never tested) that gadodiamide administered via IV injection does not diffuse into the glandular lumen. This assumption is critical because if the contrast agent is redistributed to the glandular lumen, this will represent a third compartment, and new compartmental models will be required for DCE-MRI analysis of prostate tissue. The purpose of our study was to evaluate the validity of this assumption by measuring the concentration of gadodiamide in the glandular lumens of the mouse prostate after IV injection performed with the use of x-ray fluorescence microscopy (XFM) imaging in situ [14, 15].

Materials and Methods

Animal Model

Animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Chicago. Pathogen-free C57Bl6 male mice that had a body weight of 12 g at the age of 10 weeks were purchased from Charles Rivers Laboratories. Nine C57Bl6 male

mice that were 28 weeks old underwent XFM imaging in this study. Six mice that were 28 weeks old were sacrificed 10 minutes after injection of gadodiamide (Omniscan, GE Healthcare); the mice were sacrificed by means of an overdose of isoflurane and cervical dislocation. The original stock solution of Omniscan was 287 mg/mL in aqueous solution (pH, 6.40). We diluted the solution in a 1:15 (volume/volume) ratio of Omniscan and saline (pH, 6.42). The diluted solution of Omniscan (19.13 mg/mL) was passed through a sterile 0.22- μ m filter unit (MILLEX-GP-CE0459, Millipore) at room temperature before injection. Mice were anesthetized during injection with 1–2% isoflurane. We injected an average of approximately 100 μ L of the diluted solution of Omniscan into a mouse with a body weight of 25 g in approximately 5 seconds, for a total dose of 0.13 mmol/kg. In addition, three mice were sacrificed in a similar manner after injection of 100 μ L of saline, and these three mice served as control mice.

Tissue Harvesting for Immunohistochemical Analysis and X-Ray Fluorescence Microscopy

Sections of mouse prostate (ventral and anterior) tissues were excised and embedded in optimal cutting temperature compound (Tissue-Tek, Miles) and frozen in liquid nitrogen for XFM and H and E staining. After cryosectioning, frozen 7- μ m-thick slices of prostate tissue samples were mounted on silicon nitride windows (silicon nitride membranes model 11108124, Silson) that were 2.5 $\times$ 2.5 mm in size, air dried, and kept in a desiccator until XFM studies could be performed. From tissue adjacent to the prostate tissue used to prepare the 7- μ m-thick slices for XFM studies, 5- μ m-thick slices were made for H and E staining, to facilitate lesion identification. Tissue sections for H and E staining were prepared at the Human Tissue Resource Center at the Pathology Department of the University of Chicago Medicine. All histologic images were evaluated by a pathologist who had 3 years of experience with murine prostate.

Six C57Bl6 male mice (age, 28 weeks) were sacrificed 10 minutes after receiving IV injection of gadodiamide (0.13 mmol/kg), and three mice were sacrificed after receiving a saline injection. Prostate tissue samples from each mouse were harvested and frozen, 7- μ m-thick slices were sectioned for XFM imaging with inplane resolution of 1 μ m, and adjacent 5- μ m-thick slices were sectioned for H and E staining. Elemental concentrations were determined from XFM images.

X-Ray Fluorescence Microscopy

XFM was performed at Beamline 2-ID-E at the Advanced Photon Source at Argonne National Laboratory. The x-ray microprobe used has been described elsewhere [14, 15]. Samples were mounted in a helium-filled chamber by use of a kinematic mount. Undulator-derived x-ray were monochromatized to 10 keV of incident energy by use of a single-bounce ^{111}Si monochromator and were focused to a spot size of 0.9 μ m horizontal and 0.9 μ m vertical by use of Fresnel zone plate optics. Incident flux was 10^9 photons per second. Fluorescence spectra were recorded using a Vortex ME4 silicon drift x-ray detector (SII Nano-Technology).

Rapid fly scans were performed on whole-tissue sections, typically with 5- μ m resolution and 100 ms of dwell time per pixel. Elemental maps were aligned to optical images obtained

using an optical microscope (DMi8, Leica Microsystems). Elemental images were used in conjunction with optical images to locate appropriate ROIs for high-resolution scans. For high-resolution scans, samples were raster-scanned through the focused x-ray beam with 1- μm resolution and 35-ms of dwell time per pixel. Fluorescence spectra were collected for 1 second per pixel for the entire 2.5-mm FOV.

Because the FOV of XFM is limited, and because limited time is available on the beamline at Advanced Photon Source, it was not possible to scan the entire tissue sample at a higher resolution. Therefore, smaller ROIs ($\approx 400 \times 400 \mu\text{m}$) were selected for XFM scans at high resolution (1 μm of in-plane resolution). Samples of mouse prostate tissue were selected for XFM if they contained mostly glands lined by epithelia surrounding glandular lumens.

Data Analysis

XFM data were processed using software written in Interactive Data Language (IDL 8.2.2, Exelis VIS). Quantitation and image processing were performed using MAPS software (MAPS-2003) [16]. The quantitative measurements of elemental concentrations, as determined by XFM, are well established [14]. Normalized fluorescence intensities for each element at each pixel were converted to a 2D concentration (expressed as the number of micrograms per square centimeter), by fitting spectra against the spectra obtained from thin-film standards (NBS-1832 and NBS-1833, National Institute of Standards and Technology) [17]. Using MAPS software, a radiologist with 10 years of experience in prostate imaging and a medical physicist with 5 years of experience in XFM imaging drew, in consensus, ROIs in the prostatic glandular epithelium and lumen on XFM images. Images of adjacent tissue stained with H and E guided the identification of both regions. Typical mean ($\pm$ SD) sizes of ROIs were 2718 ± 719 and $4563 \pm 1296 \mu\text{m}^2$ for epithelium and lumen, respectively. In each mouse, at least five glands lined by epithelium and lumens were selected. Average elemental gadolinium concentrations in the regions of the epithelia and lumens were determined for 32 prostatic glands in six mice that received IV injection of gadodiamide. Similarly, baseline concentrations of elemental gadolinium in ROIs containing lumens and epithelia were also determined for 15 prostatic glands in three mice that did not receive injection of gadodiamide (molecular formula = $\text{C}_{16}\text{H}_{26}\text{GdN}_5\text{O}_8 \cdot \text{H}_2\text{O}$; molecular weight = 591.66). The male mouse has a single prostate that contains numerous glands. Elemental concentrations (expressed as micrograms per centimeters squared) were determined on the basis of findings for XFM images of tissues with a slice thickness of 7 μm , taking into account the molecular mass of each element; elemental concentrations were expressed as the number of millimoles per liter. Elemental concentrations of gadolinium at base-line in the lumens and epithelia were lower by 36-to 100-fold, compared with ROIs for the same regions in mice that had received gadodiamide. A t test was performed for statistical analysis. Statistical significance was denoted by $p < 0.05$.

Results

In Table 1, we list the elemental concentrations of phosphorous, sulfur, iron, and gadolinium in the prostatic glandular epithelia and lumens in mice that were or were not administered gadodiamide. The mean concentration of elemental gadolinium at baseline (0.01 ± 0.01

mmol/L) was determined from measurements of prostatic tissue samples (both epithelium and lumen) when no gadodiamide was administered to mice ($n = 3$); this value was used to determine the measurement error. The average concentrations of elemental gadolinium in the regions of the epithelia and lumens in mice that were administered gadodiamide were 1.00 ± 0.13 and 0.36 ± 0.09 mmol/L, respectively. These values were 36- to 100-fold higher than the values for the same prostatic regions in mice that were not injected with gadodiamide ($p < 0.0001$).

Figure 1 compares ex vivo images of excised prostate tissue samples from a control mouse. The histologic image (Fig. 1A) shows a section obtained from a slice located immediately adjacent to the slice used in light microscopy and XFM examinations. The section was stained with H and E and prepared for precise image coregistration and lesion identification. The XFM image (Fig. 1C) shows the distribution of phosphorous in the same tissue shown in the light microscopic image (Fig. 1B).

Figure 2 shows light microscopy images and compares elemental distributions of phosphorous and gadolinium in tissue slices obtained from the prostate of healthy mice that were or were not administered gadodiamide. No gadolinium was detected in the prostate tissue when the mouse did not receive an injection of gadodiamide. However, gadolinium was detected in the prostate tissue on XFM images when the mouse was injected with gadodiamide 10 minutes before it was sacrificed. Light microscopy allows easy visualization of the epithelium and lumens.

Figure 3 compares high-resolution XFM images, showing elemental distributions of phosphorous and gadolinium in prostate tissue samples obtained from mice that did or did not have gadodiamide administered. No gadodiamide was detected in XFM images of prostate tissues when gadodiamide was not administered before the mouse was sacrificed. On the other hand, significant amounts of gadodiamide were seen in both the prostatic epithelia and the lumens when IV injection of gadodiamide occurred 10 minutes before the mouse was sacrificed (Fig. 3B).

Figure 4 shows the elemental distributions of gadolinium in a mouse prostate gland that contained epithelium and lumen. This tissue slice was prepared after IV administration of gadodiamide. The high-resolution XFM image shown in Figure 4B is compared with the image of an H and E-stained section (Fig. 4A) obtained from tissue adjacent to the slice obtained for XFM examination. The light microscopy image of the slice used in the XFM examination is shown in the inset of Figure 4A, for lesion identification. In Figure 4B, a significant amount of gadodiamide is seen as well as small artifacts that are visible because of cryosectioning and air drying of the tissue for imaging and histologic analysis. The fact that the epithelia and lumens are distinctly visible allowed accurate measurements of the elemental concentrations of gadolinium in these subcompartments. The sharp rim of gadodiamide in the ductal epithelium, which follows exactly the anatomy of the epithelium, indicates the high quality of the preparation. Redistribution during preparation and other preparation artifacts would have smeared out this sharp rim so that the high concentration of gadodiamide would not have precisely followed the anatomy of the ductal epithelium. The SD of the elemental concentration of gadolinium, as seen in Table 1, was relatively small;

this finding suggests that artifacts resulting from preparation are very modest. The results clearly show that IV-administered gadodiamide enters into glandular lumen of the prostate. In addition, Figures 3 and 4 show that the spatial distribution of gadodiamide is very heterogeneous in both the epithelia and lumens.

Discussion

Our results show that after IV injection of gadodiamide, gadodiamide uptake occurs inside murine prostatic glandular lumens and can be quantitatively measured using XFM. We were able to quantitatively determine elemental gadolinium distributions in mouse prostatic epithelia and lumens in situ. This information, if it can be applied in humans, can improve analysis of prostate DCE-MRI and increase diagnostic accuracy.

The concentration of gadodiamide in the glandular lumens was approximately a third of the concentration in the epithelia. Nevertheless, the average concentration (0.36 mM) is quite sufficient to cause a significant change in contrast enhancement on T1-weighted MR images, particularly in regions of the prostate where the fractional volume of the lumens is relatively large. The effect of contrast agent molecules in the lumen on image intensity may be accentuated because the native T1 value of luminal fluid is expected to be much longer than the T1 value in the epithelium and stroma. Thus, gadodiamide detected by XFM in the glandular lumens is likely to have a significant influence on results obtained by use of DCE-MRI.

An x-ray microscope uses electromagnetic radiation in the x-ray band to produce images of very small objects. X-rays cause fluorescence in most materials, and these emissions can be analyzed to determine the chemical elements of an imaged object. It is a powerful technique to map and quantify (trace) element distributions in biologic specimens. Because the energies of the x-ray fluorescence photons emitted from the probed spot are characteristic of the respective elements, they can be used to measure and quantify the elemental composition of a specimen. Spatial resolution of XFM images at the submicron level are easily achieved. XFM can be a useful technique for gaining a better understanding of the localization of various contrast agents in biologic tissue samples at the cellular and subcellular levels. Specimens that are obtained at different time points after injection of a contrast agent may also provide temporal information regarding localization of the contrast agent in the tissue.

Omniscan can cause adverse effects, including, very rarely, some serious adverse effects, particularly at higher than recommended doses [18]. Nevertheless, Omniscan has been approved by the U.S. Food and Drug Administration for use at the proper dose, has been used for the imaging of a large number of patients, and continues to be used clinically. Therefore, the results reported in this study are relevant to the interpretation of DCE-MRI images in routine clinical practice. Omniscan did not affect the kidney function of mice in the experiments reported here. Blood urea nitrogen levels showed no effects of Omniscan on kidney function, even when a high dose of contrast agent was injected every other week for 10 weeks [19].

For comparison, it will be valuable to study the distribution of other clinically used contrast agents. In general, it is assumed that low-molecular-weight contrast agents, like Omniscan, that do not bind to blood albumin and distribute passively will have approximately the same distribution in the microenvironment, although there will be small variations in capillary permeability. However, to our knowledge, there have been no direct tests of this assumption. Therefore, measurements like those described in our study are extremely important and will directly influence the clinical interpretation of data obtained from DCE-MRI. We plan to use XFM to study distributions of a number of clinically approved contrast agents in the future. However, this study is a first and valuable step toward improving our understanding of the dynamics of MRI contrast media in the microenvironment. If it turns out that the distribution of low-molecular-weight contrast agents is highly variable, depending on chemical structure, this finding will be extremely important, because it will be needed for interpretation of clinical data on the basis of the type of contrast agent used.

Uptake of gadodiamide in the glandular lumen implies that the two-compartment model, which is widely used to model the kinetics of contrast agents in patients with cancer, may not be valid for use in the prostate because exchange of contrast material into the lumen represents a third compartment. Thus, for accurate physiologic modeling, elucidation of the mechanism of exchange between the vessels and glandular lumen is essential. In addition, the heterogeneous distribution of gadodiamide in the microenvironment, as shown by XFM, poses additional challenges for modeling the dynamics of contrast agents. Despite the widespread use of the two-compartment model in DCE-MRI analysis of PCa, there are very limited data in the literature that describe the distribution of gadolinium-based agents in the prostate. In their study comparing the characteristics of stromal and glandular prostatic tissue as seen on DCE-MRI, Noworolski et al. [20] argued that because peak enhancement is higher in the stromal (or low ductal) tissues compared with the noncancerous, glandular tissues, there seems to be the presence of gadolinium-inaccessible space within the glands, likely the basement membrane surrounding the glands and ducts. On the basis of this indirect evidence, they concluded that gadolinium-based agents do not enter healthy prostatic glands. Even though our results are not consistent with this hypothesis, it may be true that gadodiamide only enters the glandular lumen at a later phase (the mice were sacrificed 10 minutes after injection of gadodiamide in our study) but not at the time of peak enhancement (as suggested by Noworolski et al.). Further studies that use XFM and vary the times of animal sacrifice after injection of contrast agents can provide more detailed information on the kinetics of the glandular uptake of gadodiamide and the distribution of gadodiamide in other components of the microenvironment.

The volume of the glandular lumen and the relative percentage of glandular lumen in the prostate gland were shown to be effective quantitative histologic biomarkers for differentiation of PCa from healthy prostatic tissue [21]. These parameters also decrease as the Gleason score of the cancer increases [21–23]. Distribution of gadodiamide into the luminal compartment may allow accurate estimation of the volume of this compartment with the use of new pharmacokinetic models and image acquisition protocols that take this fact into account. Observed early washout in PCa, compared with washout in the healthy prostate, may also be a manifestation of delayed uptake of the contrast agent by the larger volume of the glandular lumen in non-cancerous prostate glands. Future studies

investigating the uptake of gadolinium-based contrast agents in glands affected by cancer, compared with healthy prostate tissue, are needed. The lack of basal cells in the cancerous glands may potentially make the cancerous glandular lumen more permeable to gadolinium-based contrast agents, compared with healthy glands.

Our study had several limitations. First, our sample size was small; however, despite this small size, we were able to show significant accumulation of gadodiamide in the glandular lumen, compared with healthy prostate tissue. Second, the time established for sacrifice of mice after administration of the contrast agent was fixed (in this case, at 10 minutes). This limited our ability to provide temporal information on accumulation of gadodiamide in the lumen. Third, there were no cancerous glands in our study population. Therefore, we could not evaluate the glandular luminal uptake in cancerous glands and compare it with the uptake in benign glands. We are planning to extend our research in this direction.

In summary, we have shown the presence of gadodiamide in the lumens of murine prostate glands after IV injection of gadodiamide. By using XFM, we were able to quantify the presence of gadodiamide both in the epithelia and the lumens of the prostatic glands of mice. The concentrations of gadodiamide detected in the lumens are sufficient to have a significant effect on T1-weighted signal intensity. Understanding the distribution of gadolinium-based MRI contrast agents in healthy (benign) and cancerous prostates is critical to improving the sensitivity and specificity of DCE-MRI and decreasing the large variations in quantitative results currently seen, because it allows improved interpretation and modeling and aids in the development of new strategies for image acquisition. Further studies are needed to better characterize the kinetics of luminal uptake in benign and cancerous glands in both animal and human prostates.

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Fig. 1.

28-week-old pathogen-free C57Bl6 male mouse. Ex vivo images of excised prostate tissue from control mouse injected with 100 μ L of saline were compared.

A, Histologic image of section obtained from slice with thickness of approximately 5 μ m (H and E, $\times$ 20) shows mouse prostatic glands (PGs) (yellow dashed lines), prostatic urethra (PU), and seminal vesicle (SV).

B, Light microscopy image of section obtained from slice with thickness of 7 μ m and placed on 2.5 $\times$ 2.5-mm silicon nitride membrane shows PGs, PU, and SV.

C, Low-resolution x-ray fluorescence microscopy image (phosphorous map) of same slice shown in **B**. Image is scaled to minimum (0) and maximum (1) phosphorous concentrations, as denoted by color bars.

Fig. 2.

Distribution of phosphorous and gadolinium in tissue slices obtained from the prostates of two 28-week-old pathogen-free C57Bl6 male mice that were or were not administered gadodiamide.

A and B, Mouse that did not receive IV injection of gadodiamide before it was sacrificed. Light microscopic image (**A**) of tissue slices is shown. X-ray fluorescence microscopy (XFM) images (**B**) of tissue slices are shown, and individual elemental maps are labeled (P = phosphorous, Gd = gadolinium). Images are scaled to minimum (0) and maximum (1) elemental concentrations, as denoted by color bars.

C and D, Mouse that received IV injection of gadodiamide (0.13 mmol/kg) before it was sacrificed. Light microscopic image (**C**) shows tissue slices from excised prostate tissues. XFM images of tissue slices (**D**) are shown, and individual elemental maps are labeled.

Images are scaled to minimum (0) and maximum (1) elemental concentrations, as denoted by color bars.

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Fig. 3.

X-ray fluorescence microscopy images of elemental distribution of gadolinium (Gd) and phosphorous (P) in the epithelium and lumen of prostate tissue slices obtained from 28-week-old pathogen-free C57Bl6 male mice that were or were not administered gadodiamide. **A** and **B**, Prostate tissue sample from mouse that did receive injection of gadodiamide (**A**) and prostate tissue sample from mouse that received IV injection of gadodiamide (0.13 mmol/kg) before it was sacrificed (**B**) are shown. Prostatic epithelium (*yellow arrows, A and B*) and lumen (*arrowheads, A and B*) are shown. Images are scaled to minimum (0) and maximum (1) elemental concentrations, as denoted by color bars. Inset in both P maps shows light microscopy image. Accumulation of gadodiamide (*red arrow, B*) in prostatic lumen is shown.

Fig. 4.

28-week-old pathogen-free C57Bl6 male mouse that received IV injection of gadodiamide (0.13 mmol/kg) before it was sacrificed.

A and B, H and E-stained image ($\times 40$) of slice adjacent to that shown on x-ray fluorescence microscopy (XFM) image (**B**). Inset shown in **A** is light microscopy image of same slice. Gadodiamide is shown entering the glandular lumen of the prostate. Prostatic epithelium (yellow arrows) and lumen (red arrows) are shown. XFM image (**B**) shows elemental distribution of gadolinium in prostatic epithelium and lumen. Color bar corresponding to

minimum (1) and maximum elemental concentrations (1) and scale bars of 100 μm are shown.

TABLE 1

Elemental Concentrations in ROIs in the Epithelia and Lumen of Mouse Prostate Tissue, as Determined by X-Ray Fluorescence Microscopy

Gadodiamide Injection Status, Tissue Evaluated	Phosphorous	Sulfur	Iron	Gadolinium
Without injection				
Epithelium	90.87 ± 14.30	57.03 ± 7.13	0.77 ± 0.20	0.01 ± 0.01
Lumen	25.37 ± 6.01	41.43 ± 5.79	0.20 ± 0.03	0.01 ± 0.01
With injection ^a				
Epithelium	104.25 ± 12.92	61.92 ± 8.46	0.77 ± 0.18	1.00 ± 0.13
Lumen	28.13 ± 12.45	39.65 ± 5.79	0.23 ± 0.05	0.36 ± 0.09

Note—Data are mean (± SD) concentrations (mM).

^a IV injection of gadodiamide (0.13 mmol/kg).