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ABSTRACT

Objectives: X-ray iodinated contrast media have been shown to generate contrast in MR images when used with the Chemical Exchange Saturation Transfer (CEST) approach. The aim of this study is to compare contrast enhancement (CE) capabilities and perfusion estimates between radiographic molecules and a Gd-based contrast agent in two murine tumor models with different vascularization patterns.

Methods: MRI-CEST and MRI-CE T_{1w} images have been acquired in murine TS/A and 4T1 breast tumors upon sequential i.v. injection of iodinated contrast media (iodixanol, iohexol and iopamidol) and of gadoteridol. The signal enhancements observed in the two acquisition modalities have been evaluated using Pearson's correlation and the correspondence in the spatial distribution assessed by a voxelwise comparison.

Results: A significant, positive correlation has been observed between iodinated contrast media and gadoteridol for tumor contrast enhancement and perfusion values for both tumor models (r = 0.51-0.62). High spatial correlations have been observed in perfusion maps between iodinated molecules and gadoteridol (r = 0.68-0.86). Tumor parametric maps derived by iodinated contrast media and gadoteridol showed high spatial similarities.

Conclusions: A good to strong spatial correlation between tumor perfusion parameters derived from MRI-CEST and MRI-CE modalities indicates that the two procedures provide similar information.

Keywords: MRI; radiocontrast media; CEST; contrast agent; gadoteridol;

Key Points:

• Gd-based agents are the standard of reference for contrast-enhanced MRI

• Iodinated contrast media can provide MRI-CEST contrast enhancement in an animal tumor model

• Contrast enhancements were positively correlated between iodinated agents and gadoteridol

•Tumor perfusion map showed similar spatial distribution between iodinated agents and gadoteridol

•MRI-CEST with iodinated agents provide similar information to gadoteridol

Abbreviations and acronyms:

CAs: Contrast Agents

CE: Contrast Enhanced

CEST: Chemical-Exchange Saturation Transfer

FOV: Field of view

NSF: Nephrogenic Systemic Fibrosis

ROI: Region Of Interest

INTRODUCTION

Nowadays contrast-enhanced (CE) studies are an essential tool for radiologists, especially for applications in oncology, either when exploiting Gd-based agents within the MRI-CE approach, either upon the administration of iodinated radiographic agents for CT examinations [1-6].

However, in the past decade, serious clinical issues questioned the safety profiles of Gd-based CAs. The first issue linked the occurrence of nephrogenic systemic fibrosis (NSF) in patients with severe renal impairment to the administration of a Gd-based CA, resulting in a black-box warning on their use by FDA [7-9]. Gd-complexes with low thermodynamic/kinetic stability have been associated with this risk [10; 11]. More recently, a new concern has been raised upon signal hyperintensities observed in specific brain regions, surmising a possible long-term Gd deposition, despite no evidence for any clinical effect associated to Gd retention in brain have been proved yet [12-14].

As a consequence, despite the availability of Gd-based agents endowed with high stability and contrast efficiency (higher relaxivity), there is a raising issue among the CAs' developers to seek for possible replacement to these contrast agents. On this basis, it seems straightforward to search for alternatives in the field of iodinated contrast media currently used for the CT imaging modality [15]. In fact, both iodinated x-ray and gadolinium chelate CAs share similar physiochemical properties and simple two-compartment pharmacokinetics biodistribution [16]. After injection into the intravascular space, being small molecules, they leak rapidly out into the extravascular extracellular space. In addition, these agents are not metabolized and are excreted unchanged by passive glomerular filtration. As a consequence, the contrast kinetics for standard Gd-based MRI and iodinated CT contrast media are similar and theoretically both methods should give comparable results. However, the main challenge for the CT modality is the high doses of ionizing radiation required for providing sufficient spatial and temporal resolution for accurate tumor characterization, making it difficult to use repeatedly for treatment response monitoring. These limitations could be likely overcome if using the same radiographic molecules but within the MRI modality. This approach has been recently demonstrated with the iodinated x-ray agent iopamidol that can be

detected using the chemical exchange saturation transfer (CEST) technique [17-21] both at preclinical level [22; 23] and in a human volunteer with a 3T MR scanner [24]. In addition, several other radiographic molecules have been successfully shown to provide similar contrast enhancement information within the MRI-CEST technique to that commonly provided with the CT modality [25]. CEST-MRI has been proposed as a novel approach for several applications at clinical level [26-31]. A significant advantage of these agents through the CEST technique, in comparison to the CT modality and to Gd-based agents, relies on their capability to provide, in addition to tissue vascular characterization, accurate in vivo pH mapping in different tissues and pathological models [32-36].

The aim of this work was to determine whether the contrast enhancement capabilities and perfusion estimates generated from three iodinated contrast media (two non-ionic monomers: iohexol and iopamidol; one non-ionic dimer: iodixanol) are comparable with those provided with a Gd-based contrast agent, gadoteridol, acquired in the same tumor bearing mice upon sequential injection. Furthermore, correlation of contrast enhancements and perfusion estimates between MRI-CEST and MRI-CE images was assessed both as mean estimates in tumor regions and through a direct spatial (voxelwise) comparison of the obtained parametric images in two tumor models with different vascularization patterns.

METHODS

Chemicals

Iopamidol (Isovue®) and gadoteridol (ProHance®) were generously provided by Bracco Imaging S.p.A. (Colleretto Giacosa, Italy). Iohexol (Omnipaque® - GE Healthcare, Milan, Italy), and Iodixanol (Visipaque® - GE Healthcare, Milan, Italy) were obtained from commercial sources. All other chemicals were purchased from Sigma–Aldrich (Milan, Italy).

Animal studies

All animal procedures and husbandry were performed in accordance with our University Ethical Committee and European guidelines under directive 2010/63. Male and female BALB/C mice (Charles River Laboratories Italia S.r.l., Calco, Italy) were 8 to 10 week of age and their weights were 22 to 28 g. $2.5x10^5$ TS/A cells (HER2 positive breast adenocarcinoma) or $4.0x10^5$ 4T1 cells were subcutaneously inoculated into both flanks of BALB/c mice. For the TS/A group, thirty-three mice (n=11 mice for each investigated iodinated molecules) were used, for a total of 63 tumors (3 tumors did not grow). For the 4T1 group, thirty mice (n=10 mice for each investigated iodinated molecules) were used, for a total of 60 tumors. Mice received the same dose of iodinated contrast media of 4 g Iodine / kg body weight, followed, 30 minutes later, by a 0.1 mmol Gd / kg body weight injection of gadoteridol, slowly injected via the same catheter without removing the animal from the MRI scanner.

In vivo MR imaging

MR images were acquired on a Bruker Avance 7T MRI scanner (Bruker BioSpin MRI Ettlingen, Germany) equipped with a micro 2.5 MICWB 30mm quadrature (1H) imaging probe. Mice were anesthetized by injecting a mixture of xylazine 5 mg/kg (Rompun, Bayer, Milan, Italy) and tiletamine/zolazepam 20 mg/kg (Zoletil 100, Virbac, Milan, Italy). Respiratory rate was continuously monitored using a respiratory air pillow (SA Instruments, Stony Brook, NY).

After acquisition of scout images and of a T_2-weighted anatomical reference image, Z-spectra before and after iodinated contrast media injection were acquired in the frequency offset range ±10 ppm using a single-shot RARE sequence with centric encoding (typical setting TR/TE/NEX = 6.0 s/2.7 ms/1) preceded by a 3μT cw block presaturation pulse for 5 s and by a fat-suppression module. We used an acquisition matrix of 96x96 for a field of view of 3x3 cm (in-plane spatial resolution = 312.5 μm) with a slice thickness of 1.5 mm. 30 minutes after the last Z-spectrum acquisition, T_1-weighted images before and after gadoteridol injection were acquired using an axial 2D fast low angle shot (FLASH) gradient echo sequence (TR 70 ms; TE 1.5 ms; flip angle 45°; slice thickness

1.5 mm; FOV 30 mm; matrix 96 × 96; 50 averages) keeping the same geometry, orientation and spatial resolution of the CEST images.

Data analysis

All MRI-CEST and T_{1w} images were analyzed using home-made scripts implemented in MATLAB (The Mathworks, Inc., Natick, MA, USA). The Z-spectra were interpolated, on a voxel-by-voxel basis, by smoothing splines, B_0-shift corrected and saturation transfer efficiency (ST%) was measured by punctual analysis [37]. For in vivo images, difference contrast maps (ΔST%) were calculated by subtracting the ST contrast observed after iodinated contrast media injection from the ST contrast observed before the injection on a per voxel basis in order to reduce the confounding effect of the endogenous CEST contributions.

T_{1w} images were analyzed on a voxel-by-voxel basis and signal intensities enhancement (SIenh%) was measured between pre- and post- injection of gadoteridol.

The extravasation fraction (perfusion) estimates were calculated for each agent as the percentage of pixels showing a ΔST% or a SIenh% above the threshold (3% and 30% for CEST and T_{1w} images, respectively) in the manually-defined tumor region of interest (ROI) [38].

Reproducibility study

For reproducibility studies, a group of TS/A tumor bearing mice (n=8) were imaged in two consecutive days following administration of iopamidol or of gadoteridol. Contrast enhancement and extravasation fraction estimates obtained by iopamidol or by gadoteridol were compared.

Statistical analysis

All results were expressed as mean ± standard deviation (SD). The statistical significance of the differences between the means of contrast enhancement and perfusion values was calculated using one-way ANOVA followed by the Bonferroni post-hoc test. The Pearson's correlation coefficient (r)

between the mean values in the tumor ROIs was calculated to assess the relationship between the obtained CEST and Gd-based estimates and reported with the 95% confidence intervals (CI). Correlations were considered low when $0.3 < r < 0.5$, moderate when $0.5 < r < 0.7$ and strong when $0.7 < r < 1$ [39]. Intermethod agreement between CEST- and Gd-based estimates was further examined by using Bland-Altman analysis.

Voxelwise spatial correlation between enhanced images and between extravasation parametric maps obtained from CEST and Gd-based images were measured in tumor ROIs by calculating the two-dimensional correlation coefficient in the same tumor slice. The percentage of spatial similarity between extravasation fraction images was calculated in tumor ROIs by counting pixels where both contrast agents have been detected above their respective threshold. Spatial similarity maps have been color-coded as follows: pixels where both contrast agents have been detected as blue pixels, pixels in which only CEST contrast agent was detected as red pixels and pixels where only Gd-based contrast agent was detected as green pixels. Spatial correlation estimates are reported as mean ±SD.

The calculations were performed with GraphPad Prism (GraphPad Software, La Jolla, CA).

For test-retest reliability experiment, Intraclass correlation (ICC) coefficient along with 95% confident interval was computed. P values less than 0.05 were considered statistically significant.

RESULTS

Reproducibility of CEST and T_{1w}-CE imaging

Fig. S1 shows contrast enhancement and extravasation fraction values in test-retest experiment following iopamidol or gadoteridol injection. High ICC coefficients were observed for contrast enhancement values upon iopamidol or gadoteridol injections (0.80 and 0.78 for iopamidol and gadoteridol, respectively). Similar agreement were also observed for the extravasation fraction estimates (ICC= 0.73 and 0.74 for iopamidol and gadoteridol, respectively).

Comparison of tumor perfusion assessment in the TS/A tumor model

In all tumor bearing mice, contrast enhancements upon i.v. injection of the investigated iodinated molecules were successfully observed. Fig. 1 shows representative whole tumor ROI Z-spectra for the CEST iodinated molecules before and after the injection in the TS/A tumor model. A marked increase of the CEST saturation transfer effect (ST) is visible within the tumor region, upon the injection, for all the investigated x-ray molecules from the baseline ST curves. The gadoteridol dose was administered 30 minutes after the acquisition of CEST-iodinated scans using the same catheter and without removing the animal from the scanner, to maintain the same position in order to proceed with the subsequent voxelwise comparison of the CEST and Gd-based-derived parameters.

The three iodinated contrast media showed significant variability in the observed enhancement values in the corresponding MRI-CEST images. The mean CEST contrast enhancement (ΔST%) measured inside the tumor ROIs were 4.4 ±0.2, 5.5 ±0.4 and 6.4 ±0.2 for iodixanol, iohexol and iopamidol, respectively (Fig. 2a), with significant differences between iodixanol and iopamidol ($P<0.001$). Conversely, the mean contrast enhancements upon gadoteridol injection (ΔSI%) were similar for all the investigated tumors, with an overall average value of 62 ±5% (Fig. 2b).

The mean extravasation fractions measured as the percentage of voxels where contrast agent detection was above the threshold were 0.61 ±0.02, 0.73 ±0.03 and 0.79 ±0.02 for iodixanol, iohexol and iopamidol, respectively (Fig. 2c). The mean perfusion values measured upon gadoteridol injection were in the range 0.79 to 0.89, not statistically significant between the three sets of animals (as grouped on the basis of the administered iodinated molecules, Fig. 2d).

Fig. 3 shows the scatterplot of the CEST-iodinated derived parameters versus CE-gadoteridol derived parameters for the 63 matched tumors. The correlation of the mean parameters between the two imaging approaches were moderate for contrast enhancement ($r= 0.51$, CI: 0.30-0.67, Fig. 3a) and perfusion ($r=0.62$, CI: 0.42-0.74, Fig. 3b), respectively.

In Bland-Altman analysis (Fig 3c) CEST method underestimated the extravasation fraction by a mean of -0.2 with a lower limit of −0.48 (− 1.96SD) and an upper limit of 0.12 (1.96SD).

Representative parametric maps for the three investigated iodinated molecules are shown in Fig. 4, as CEST contrast enhancement maps (Fig. 4a) and T_{1w}-gadoteridol enhancement maps (Fig. 4b), respectively, overimposed onto the anatomical image. Corresponding T_{1w} contrast enhanced images acquired after gadoteridol injection are shown in Fig S2. The similarity in the distribution of the two classes of agents yields an overall good spatial correspondence between the two MRI-based approaches, with similar contrast enhancements values throughout the entire tumor.

A strong spatial correlation was observed, with mean spatial correlation values of 0.75 ±0.06, 0.79 ±0.05 and 0.83 ±0.06 for iodixanol, iohexol and iopamidol, respectively (Fig. 5a). Iopamidol showed a higher spatial correlation for the enhancement maps with gadoteridol in comparison to iodixanol.

The spatial correlation of the extravasation maps between iodinated and gadoteridol molecules was also strong, with mean values of 0.68 ±0.08, 0.80 ±0.09 and 0.86 ±0.05 for iodixanol, iohexol and iopamidol, respectively (Fig. 5b).

Fig. 5c shows the percentage of spatial equivalence (similarity index), calculated as the number of pixels where both contrast agents have been detected. A much higher similarity index for iopamidol and iohexol (similarity mean values of 0.64 ±0.10 and 0.76 ±0.16, respectively), in comparison to iodixanol (0.47 ±0.08) was observed. Representative similarity maps with color-coded pixels are shown in Fig. 4c. For both iopamidol and iohexol in almost all voxels both the iodinated and the gadoteridol molecules have been detected.

Comparison of tumor perfusion assessment in the 4T1 tumor model

In the 4T1 tumor model similar contrast enhancement values were observed in comparison to the TS/A tumor model. The mean CEST contrast enhancement (ΔST%) measured inside the tumor ROIs were 4.0 ±0.2, 4.8 ±0.2 and 5.8 ±0.3 for iodixanol, iohexol and iopamidol, respectively (Fig. S3a). Also mean contrast enhancement values upon gadoteridol injection (ΔSI%) were similar for all the investigated tumors, with an overall average value of 68 ±4% (Fig. S3c).

The observed extravasation was reduced in comparison to the TS/A tumor model for both the iodinated molecules (range: 0.55-0.70, Fig. S3b) and for gadoteridol (range 0.55 to 0.70, Fig. S3d). The correlation of the mean parameters between the two imaging approaches for the 60 matched tumors were moderate for contrast enhancement (r= 0.51, CI: 0.30-0.68, Fig. 3d) and perfusion (r=0.56 CI: 0.36-0.71, Fig. 3e), respectively. The Bland–Altman analysis (Fig. 3f) yields a mean difference for perfusion estimation of -0.06 (limits of agreement: -0.21, 0.33).

A different vascularization pattern is evident in the contrast enhancement maps, showing a clear reduction in detection for all the contrast agents when moving from the tumor rim to the tumor core region (Fig. 6 and Fig. S4).

The similarity in the distribution of the two classes of agents yields an overall good spatial correspondence between the two MRI-based approaches, in particular in the rim tumor regions that present higher contrast enhancement.

A strong spatial correlation was observed, with mean spatial correlation values for the enhancement maps of 0.72 ±0.05, 0.79 ±0.05 and 0.82 ±0.05 for iodixanol, iohexol and iopamidol, respectively (Fig. 5d). The spatial correlation of the extravasation maps between iodinated and gadoteridol molecules was also strong, with mean values of 0.64 ±0.06, 0.72 ±0.08 and 0.86 ±0.07 for iodixanol, iohexol and iopamidol, respectively (Fig. 5e). High similarity index were observed for all the three investigated molecules: 0.70 ±0.09, 0.64 ±0.08 and 0.58 ±0.09 for iopamidol, iohexol and iodixanol, respectively (Fig. 5f). Representative similarity maps with color-coded pixels are shown in Fig. 6.

DISCUSSION

To our knowledge, this is the first study comparing MRI-CEST with conventional MRI-CE in the assessment of tumor contrast enhancement and perfusion properties in experimental mouse tumor models. The sequential injection in the same animal of one iodinated CEST agent and one Gd-based agent allows ruling out any bias concerning position and spatial resolution between the two approaches, thus making possible a precise comparison of the parameters obtained by the two

techniques. Our study demonstrated that the contrast enhancement values derived from the MRI-CEST approach (with iodinated molecules) is comparable to that derived from MRI-CE (with gadoteridol). In addition, mean tumor perfusion values were significantly correlated and parametric images showed high spatial similarity in two tumor models with different vascularization properties. Therefore, iodinated contrast media within the MRI-CEST modality can represent suitable candidates as alternative agents to Gd-based ones for the in vivo evaluation of tumor properties.

Contrast agents are routinely used to detect tumors either in terms of an enhanced vascularization or in terms of an extensive extravasation [40; 41]. Overall, all the investigated radiographic molecules were able to generate sufficient MRI-CEST contrast to be detected in the tumor region. Iohexol and iopamidol showed comparable contrast enhancement efficiency, in contrast iodixanol showed reduced contrast enhanced values (Fig. 1). The radiologic efficacy of a contrast agent can be expressed by its increase in signal intensity (enhancement). Gd-based agents provide the highest tumor contrast enhancements, whereas iodinated molecules show a lower degree of enhancement when used within the CT or, as in this work, in the MRI-CEST modality [42]. In this study, the obtained mean tumor contrast enhancement by using iodinated contrast media with the MRI-CEST technique is in the range of 4-10%. Despite this value is lower of that measured with gadoteridol (60%), it is still comparable to that attainable in tumor patients using the CT modality with the same agents [43] and higher to what attainable with biodegradable glucose-based systems [44; 45]. MR imaging is considered superior to CT in tissue characterization because of its superior contrast resolution in both unenhanced and conventional T_{1w} contrast-enhanced images. On the other hand, the linear relationship between the iodinated CAs concentration and the measured density (in Hounsfield unit) are significant advantages of the CT modality that are counterbalanced by limited tissue coverage and radiation burden. Thus, iodinated agents combined with the MRI-CEST technique may be a useful tool for the noninvasive characterization of tumor perfusion, with the advantage that this method does not involve any ionizing radiation exposure.

Tumor tissue is strongly heterogeneous, therefore the exploited imaging approach should provide enough spatially resolved resolution for detecting both hypervascular (related to angiogenesis) and hypovascular regions. MRI with Gd-based agents clearly assess the heterogeneous distribution of microcirculation properties, as shown in Fig. 4b and Fig 6b, where areas of high intensity are well detected in the tumor rim. The 4T1 tumor model showed a more heterogeneous vascularization in contrast to the TS/A one, whose vessel distribution is more homogeneous. The particular vascularization pattern for 4T1 tumor model with a strong enhanced tumor rim and a less or not-enhanced tumor core was already been observed elsewhere [46; 47]. Notably, similar information was provided by iodinated contrast media, with areas of higher and lower contrast enhancement that spatially parallel those obtained with gadoteridol (Fig. 4a and Fig. 6a). Though the Pearson's correlation showed a good correlation between MRI-CEST and MRI-CE parameters, it does not take spatial information into account. Therefore, we calculated the spatial correlation for assessing similarity in terms of enhancement and perfusion estimates on a voxel-by-voxel basis upon the sequential injection of the iodinated and Gd-based agents. Interestingly, all the investigated contrast media provided moderate to strong spatial correlation, with iohexol and iopamidol showing higher spatial correlation with parametric maps derived from gadoteridol in comparison to iodixanol. One possible explanation accounting for the observed behavior may be ascribed to the different molecular weight of the contrast agents, with iodixanol being three times larger than gadoteridol. Concurrently, the higher molecular weight of iodixanol may result in slower wash-in kinetic and tumor accumulation, hence in reduced contrast enhancement.

Another interesting issue is the slightly higher extravasation fraction of gadoteridol (55-90%) in comparison with that of iodinated contrast media (55-80%). In principle the enhancement factor of an injected contrast agent inside the tumor pixels is the result of both accumulation and contrast efficiency. Because of its relatively low viscosity, gadoteridol can more easily enter extravascular/extracellular spaces, thus yielding a higher tumor perfusion [48]. Another explanation could be that Gd-based agents possess intrinsic higher contrast efficiency [49]. As a consequence,

even small amounts of Gd-chelates are sufficient to provide enough contrast enhancements; conversely, iodinated contrast media require sustained accumulation. However, no significant differences were found between the two classes of CAs in terms of extravasation fraction values.

Our study is hampered by some limitations. The wash-in/wash-out properties of the iodinated contrast agents may affect the gadoteridol-derived estimates due to incomplete differential clearance of the first administered contrast agent. Tentatively, these effects can be ignored, considering that (i) iodinated contrast media are low-molecular-weight extracellular contrast agent with fast elimination kinetics, (ii) enough time was allowed for the wash-out of the iodinated compounds before gadoteridol injection and (iii) pre-injection T_{1w} images have been acquired to minimize such confounding effects. This was demonstrated by the fact that the extravasation fraction estimated from the T_{1w} images was not affected by the order of injection of the contrast agents (Fig. S5). In fact, no significant differences were observed in the extravasation fraction when gadoteridol was injected before or after iopamidol (P=0.4). In addition, also the extravasation fraction measured from iopamidol was independent of the contrast agent injection sequence (P=0.15).

Another important point is that small changes in temperature during the examination may influence tumor physiology, hence the calculated perfusion estimates. However, we observed relatively small changes in temperature between sequential CEST and T_{1w} image acquisitions ($\Delta T < 2°C$, Fig. S6), therefore we can reasonably assume that the perfusion estimates have not been significantly affected.

Reduction in r_{1p} values at 7T compared to at lower fields, as those clinically available, could have resulted in lower contrast enhancement values for gadoteridol [50].

In conclusion, a good correlation between contrast enhanced images obtained using radiographic molecules (MRI-CEST) and gadoteridol (MRI-CE) was found. Voxelwise comparison indicated high spatial distribution similarity of contrast enhancement and perfusion maps. These results demonstrate the potential of MRI-CEST using clinically available x-ray CAs in the set-up of tumor

MRI-CE procedures, as those available using conventional Gd-based agents, although further work appear still necessary to improve CEST contrast efficiency.

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FIGURE LEGENDS

Figure 1. Representative whole tumor ROI Z-spectra (a) and corresponding CEST contrast ST spectra (b) acquired before and after i.v. injection of the investigated iodinated contrast media (iodixanol, iohexol and iopamidol, from top to bottom) for the right tumor of mice shown in Fig. 4.

Figure 2. Descriptive statistics of the contrast enhancement (a) and extravasation fraction (b) upon sequential i.v. injection of one of the three investigated iodinated molecules within the MRI-CEST approach and of contrast enhancement (c) and extravasation fraction (d) after gadoteridol injection within the MRI-T_{1w} approach measured in ROIs encompassing the TS/A tumor tissues.

Figure 3. Scatterplot of MRI-CEST derived parameters versus MRI-gadoteridol derived parameters for contrast enhancements (a) and extravasation fractions (b) for the 63 matched TS/A tumor ROIs and for contrast enhancements (d) and extravasation fractions (e) for the 60 matched 4T1 tumor ROIs. Data have been linearly fitted and Pearson's correlation coefficient is additionally stated in each plot. Bland-Altman plots of the extravasation fraction agreement for TS/A (c) and 4T1 (f) tumors: the solid lines and dashed lines indicate the mean difference and the 95% (1.96SD) quantile limits of the parameters derived from the two contrast based approaches.

Figure 4. Representative contrast enhanced maps upon (a) i.v. injection of iodinated molecules (iodixanol, iohexol and iopamidol, from left to right) as MRI-CEST ΔST% maps (calculated as ST% post injection – ST% pre injection), followed by i.v. injection of gadoteridol (b) as ΔSI% maps (calculated as SIpost-injection – SIpre-injection / SIpre-injection), superimposed onto anatomical T_{2w} images in TS/A breast tumors. Example of similarity analysis (c) showing pixels where both iodinated molecules and gadoteridol have been detected (blue pixels) or when only one contrast agent has been detected (red and green, for iodinated or gadoteridol, respectively).

Figure 5. Box-plots for spatial voxel-wise correlation comparing parametric maps derived using iodinated contrast media with gadoteridol of contrast enhancements (a), extravasation fraction (b) and similarity percentage (c) for the TS/A tumor model and of contrast enhancements (d), extravasation fraction (e) and similarity percentage (f) for the 4T1 tumor model. The central mark is the median, the edges of the boxes are the 25^{th} and 75^{th} percentiles and the range of the whiskers includes 5 to 95 percentiles of the data.

Figure 6. Representative contrast enhanced maps upon (a) i.v. injection of iodinated molecules (iodixanol, iohexol and iopamidol, from left to right) as MRI-CEST ΔST% maps (calculated as ST% post injection – ST% pre injection), followed by i.v. injection of gadoteridol (b) as ΔSI% maps (calculated as SIpost-injection – SIpre-injection / SIpre-injection), superimposed onto anatomical T_{2w} images in 4T1 breast tumors. Example of similarity analysis (c) showing pixels where both iodinated molecules and gadoteridol have been detected (blue pixels) or when only one contrast agent has been detected (red and green, for iodinated or gadoteridol, respectively).