RESEARCH ARTICLE

Cerebral blood perfusion deficits using dynamic susceptibility contrast MRI with gadolinium chelates in rats with post-ischemic reperfusion without significant dynamic contrast-enhanced MRI-derived vessel permeabilities: A cautionary note

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Abstract

In this study, we quantified perfusion deficits using dynamic susceptibility contrast magnetic resonance imaging (DSC-MRI) with an extravasating contrast agent (CA). We also investigated the efficacy of leakage compensation from CA pre-load in brains from post-ischemic rat models without significant dynamic contrast-enhanced MRI-derived vessel wall permeability. DSC measurements were obtained using fast (0.3 s) echo-planar imaging in both normal rats and rats with transient middle carotid artery occlusion (MCAO) (1-h MCAO, 24-h reperfusion) after successive administrations of gadoterate meglumine (Dotarem) and intravascular superparamagnetic iron oxide nanoparticles (SPION). The relative cerebral blood volume (CBV) and cerebral blood flow (CBF) values acquired using Dotarem were significantly underestimated (~20%) when compared to those acquired using SPION in ipsilesional post-ischemic brain regions. A slight overestimation of relative mean transit time was observed. Areas with underestimated CBV and CBF values from the corresponding error maps encompassed the area of infarcted tissue (apparent diffusion coefficient < 500 μm²/s) and mostly coincided with the area wherein conspicuous longitudinal relaxation time differences were observed pre- vs. post-injection of Dotarem. The DSC measurements with significant pre-load (0.3 mmol kg⁻¹) of Dotarem displayed minimal perfusion deficits when compared to those determined using the reference intravascular SPION.

Introduction

Post-ischemic reperfusion measurements in small animal models provide useful information for the optimization of intervention therapies and the evaluation of prognostic assessments [1,2]. As brain perfusion provides important information regarding the functional status of
stroke lesions, several investigations have been carried out to develop accurate magnetic resonance (MR) measurements and delineation strategies for post-ischemic hypo- and hyper-perfused lesions [3,4]. Dynamic susceptibility contrast MR imaging (DSC-MRI) provides highly sensitive perfusion information and can be used to estimate the exogenous tracer concentration. Thus, it can also be used to extract information regarding cerebral perfusion, including cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT), based on drug tracer models [5–7]. Typically, such pharmacokinetic models assume that the bolus-injected contrast agent (CA) does not extravasate and that the underlying blood vessels are randomly oriented. These are reasonable assumptions for a normal brain with an intact blood-brain barrier (BBB) when using gadoterate meglumine (Dotarem, Guerbet). However, when the BBB is disrupted (e.g., due to the presence of tumors or stroke), Dotarem, which is a member of the most commonly used family of MRI CAs, leaks through vessel walls. Consequently, in the presence of a damaged BBB, the DSC-MRI signal is significantly different from that calculated based on the no-leak assumption. Several studies have reported apparent DSC-MRI signal underestimations and correction methods for extravasating CAs used to image tumors with leaky vessels [8–13].

In studies of brains with early (<24 h) post-ischemic reperfusion and relatively small BBB leakage (insignificant $K^\text{trans}$ obtained based on DCE-MRI [14]), the resulting DSC-MRI perfusion deficits are sometimes overlooked. In addition, the efficacy of leakage compensation using CA pre-load has not been directly quantified in a post-ischemic animal model. Systematic underestimations of cerebral perfusion parameters derived using DSC-MRI are problematic, especially for longitudinal animal studies, even if they are much smaller than those observed in cases of leaky tumors. This is because the DSC-MRI findings will result in ambiguity in the CBF and CBV threshold criteria used to distinguish the normal and hyper-perfused areas of the lesion. A previous study compared leaky vessel-derived CBF values obtained using DSC-MRI to those obtained using another MRI modality, namely, arterial spin labeling, in a stroke model [15]. However, these values were not compared to those obtained using an intravascular CA.

Accurate monitoring of first-pass (0–4 s in rat, as shown in S1 Fig) DSC time curves requires fast acquisition methods, such as echo-planar imaging (EPI), for application in animal models. This renders DSC-MRI signals much more sensitive to $T_1$ and susceptibility changes due to CA leakage in animal model studies. Post-ischemic hyper-perfusion (~1 day) was frequently reported in the 1-hour middle cerebral artery occlusion (MCAO) animal model, which potentially has compromised BBB integrity in post-ischemic brain regions [3]. Therefore, a systematic comparison between dual DSC-MRI methods using extravasating and intravascular CAs in this model may be used to quantify and localize the perfusion errors and efficacy of CA pre-load in brains subjected to post-ischemic reperfusion.

In this study, an additional intravascular SPION measurement served as a reference to eliminate the confounding factor of vessel wall permeability following fast (repetition time [TR] = 0.3 s) DSC-MRI acquisition with EPI using Dotarem [13]. The DSC-MRI time curves obtained using either Dotarem or SPION were then directly compared for both normal rats and rats subjected to transient (1-hour) middle carotid artery occlusion (tMCAO) surgery following 1-day reperfusion. The cerebral blood perfusion underestimation errors were then quantified. The measured perfusion underestimation errors for both CAs and the apparent diffusion coefficient (ADC) values were used to segment the ipsi-stroke hemisphere. Cerebral perfusion errors, ADCs, and relative CBV values were compared among the infarct, peri-infarct, and normal zones. The efficacy of leakage compensation due to CA pre-load in brains from post-ischemic rat models was then studied using fast DSC-MRI acquisitions.
Methods
Animal preparation
The experiments were approved by the Institutional Animal Care and Use Committee of Ulsan National University of Science and Technology. Female Sprague-Dawley rats (SD; weight: 150–250 g) were obtained from Orient Bio (Gyeonggi, Republic of Korea). The SD rats were anesthetized with isoflurane during the MRI scan. To compare the two different DSC-MRI derived perfusion maps, rats in the normal (n = 3) and stroke (n = 6) groups were injected twice with the different CAs (Dotarem and SPION), as shown in S2 Fig. Two Dotarem injections were also performed in normal rats (n = 3). For both leakage compensation and vessel wall permeability estimations, DCE-MRI acquisition was performed before the two DSC-MRI acquisitions in the additional stroke group (n = 3). Rats in the stroke group were subjected to 1-h intraluminal mono filament (0.35 mm diameter filament, Doccol Corporation, USA) MCAO followed by 1-day reperfusion.

Magnetic resonance imaging
All studies were performed using a 7-T MR scanner (Bruker, Germany) with a 40-mm volume coil and a surface coil. Dotarem and SPION were injected to evaluate the area under the curve (AUC) values of the DSC signals for the extravasating and intravascular CAs, respectively. AUC values for successive Dotarem-Dotarem injections were obtained in the control experiments using normal rats (n = 3). Corresponding values for successive Dotarem-SPION injections were also obtained (n = 3). For the tMCAO model, AUC values for successive Dotarem-SPION injections were obtained in rats subjected to stroke and reperfusion (n = 6). To identify regions of damaged BBB in the rats subjected to stroke, we obtained pre- and post-injection $T_1$ maps. ADC maps and $T_2$-weighted images were also obtained for rats subjected to stroke. All of the MRI procedures are detailed in S2 Fig. For both leakage compensation and vessel wall permeability estimations, the DCE-MRI acquisitions were performed before the two DSC-MRI acquisitions (Dotarem-SPION injections) in the additional stroke group (n = 3).

The DSC-MRI perfusion maps were acquired using a gradient-echo EPI sequence with the following pulse sequence parameters: TR = 300 ms, effective echo time (TE) = 17 ms, field of view (FOV) = $30 \times 30$ mm$^2$, matrix size = $96 \times 96$, number of slices = 3, number of averages (NA) = 1, slice gap = 0.2 mm, slice thickness = 1 mm, bandwidth = $3.5 \times 10^5$ Hz, number of segments = 1, flip angle = 35˚, and temporal resolution = 0.3 s. The Dotarem and SPION injection doses were 0.3 mmol·kg$^{-1}$ (130 μl) and 0.075 mmol·kg$^{-1}$ (75 μl), respectively.

The $T_1$ maps were obtained using rapid acquisition with relaxation enhancement (RARE) with variable TR (RAREVTR [16]) with the following parameters: TR = 80, 150, 200, 400, 800, 1200, 1600, 2000, 2500, 3000, and 4500 ms; RARE factor = 4; effective TE = 4.58 ms; NA = 1; FOV = $30 \times 30$ mm$^2$; matrix size = $96 \times 96$; number of slices = 3; slice gap = 0.2 mm; and slice thickness = 1 mm.

The ADC maps were acquired using diffusion-weighted EPI with the following parameters: TR = 5000 ms; number of segments = 4; effective TE = 20 ms; b-values = 200, 400, 600, and 1000 s·mm$^{-2}$; NA = 1; FOV = $30 \times 30$ mm$^2$; matrix size = $96 \times 96$; number of slices = 3; slice gap = 0.2 mm; and slice thickness = 1 mm. Three ADC maps along the x, y, and z directions were averaged to obtain trace ADC values.

The $T_2$-weighted images were obtained using RARE [17] with the following parameters: TR = 5000 ms, RARE factor = 4, effective TE = 30 ms, NA = 2, FOV = $30 \times 30$ mm$^2$, matrix size = $256 \times 256$, number of slices = 20, slice gap = 0 mm, and slice thickness = 0.5 mm.
DCE-MRI data were acquired using fast low angle shot [18] with the following parameters: TR = 35 ms, TE = 1.9 ms, NA = 1, FOV = 30 × 30 mm², matrix size = 96 × 96, number of slices = 3, slice thickness = 1 mm, number of repetitions = 180, temporal resolution = 3.36 s, and flip angle = 30°. The Dotarem injection dose for DCE-MRI was 0.1 mmol·kg⁻¹. The Dotarem injection was followed by a 0.1–0.2 mmol·kg⁻¹ flush (after 15 min) after the acquisition in studies of leakage compensation with CA pre-load for the subsequent fast DSC-MRI [13].

Data analysis

For the in vivo study, two relative CBV (rCBV) maps for the first and second CA administrations were estimated from the AUC measurements from 0 to 4 s. The equation \( \frac{\Delta R^*_S(t)}{T_E} = -\frac{1}{T_E} \ln \left( \frac{S_{pre}(t)}{S_{post}(t)} \right) \) should capture the first-passage of CA, as shown in S1 Fig. Both rCBV maps acquired before the normalization process are shown in S3 Fig. Because the two AUC maps contained relative values, the AUC map of the first administration (Dotarem) was normalized to that of the second administration (SPION) for the region with an intact BBB. For the normalization, the intact-BBB region was drawn manually on the right (normal group) or the BBB-disrupted region (stroke group) hemisphere. As shown in S3 Fig, scatterplots and linear fittings were performed for both AUC maps. Because the two AUC maps contained relative values, the AUC map of the first administration (Dotarem) was normalized to that of the second administration (SPION) for the region with an intact BBB. For the second injection, the corresponding normalized AUC_Dotarem and AUC_SPION values for the intact-BBB region. The AUC_Dotarem map obtained after the first injection was then divided based on the slope of the regressed line for the intact-BBB region. The corresponding normalized AUC_Dotarem map was referred to as rCBV_Dotarem. Similarly, the AUC_SPION map was referred to as rCBV_SPION. Following the above normalization procedure, the AUC ratio between the first and second injections was always 1 by definition (\( y = x \)) for intact-BBB regions, as shown in S3 Fig. Consequently, the rCBV_Dotarem values for the stroke region can be directly compared to those in the reference rCBV_SPION, rCBF_SPION, rCBF_Dotarem, the relative MTT for SPION (rMTT_SPION), and rMTT_Dotarem were also calculated. The rCBV_error map was computed by dividing the rCBV_SPION map by the rCBV_Dotarem map. We used a similar procedure to generate the rCBF_error and rMTT_error maps. For the statistical comparisons, a region of interest (ROI) was defined in the left hemisphere (normal group) or the BBB-disrupted region (stroke group). For the stroke group, the BBB-disrupted region was identified using the T₁ difference map, which illustrates the difference between the T₁ maps obtained before and after the Dotarem injection.

Voxel-wise ADC values \( (S = S_0 e^{-ADC \times B}) \) were estimated for three gradient directions and averaged to obtain the ADC map. Areas of infarction (ADC < 500 µm²/s), peri-infarction with BBB damage (ADC > 500 µm²/s, rCBV_error > threshold), and normal tissue (ADC > 500 µm²/s, rCBV_error < threshold) were segmented by thresholding each value correspondingly in the ipsi-stroke hemisphere [19]. The threshold value was equal to \( (\text{mean}_{rCBV_{error}}^{\text{contra.}} + \text{std}_{rCBV_{error}}^{\text{contra.}}) \), where mean_{rCBV_{error}}^{\text{contra.}} and std_{rCBV_{error}}^{\text{contra.}} were the average value and standard deviation of rCBV_error in the intact contralateral brain hemisphere, respectively. Histograms of the ADC, rCBV_error, and rCBV_SPION \( (V_p) \) values for the corresponding areas were then generated for use in the characterizations.

For the vessel wall permeability estimation, the DCE-MRI time curves were converted to ΔR₁ values and fitted using the extended Toft model [20] to estimate \( K^\text{trans} \), \( V_e \), and \( V_p \). Pre- and post-injection \( T_1 \) values were also sequentially measured in order to enable longitudinal monitoring of the leakage of Dotarem. The efficacy of leakage compensation using CA pre-load (0.1 mmol·kg⁻¹ for DCE-MRI followed by a 0.1–0.2 mmol·kg⁻¹ flush) was investigated by comparing the rCBV maps for the subsequent Dotarem- and SPION-derived DSC-MRI data.
Results

Extravasating (Dotarem) and intravascular (SPION) CAs in the 1-h MCAO 1-day reperfusion rat model

To evaluate the extent of extravasation for both Dotarem and SPION injections, $T_1$ maps were measured before and after the CA injections. As shown in S4 Fig, the $T_1$ values of the infarction region (ADC < 500 μm$^2$/s) were significantly lower than those of the contralateral region (Wilcoxon rank sum test, p < 0.01, p = 0.001) after the Dotarem injection (0.3 mmol/kg) in the tMCAO model. In contrast, no significant differences were observed between the pre- and post-injection $T_1$ maps (Wilcoxon rank sum test, p > 0.05, p = 0.229) after the SPION injection, indicating the absence of significant leakage of SPION at this time point. Note that the large-vessel region of the contralateral hemisphere was avoided when manually drawing the ROI because this region exhibited a shortened $T_1$ value even though the CA was not extravasated. The same ROI was used to compare the Dotarem and SPION results. Statistical analysis of the differences in $T_1$ measurements pre- vs. post-SPION injection confirmed that SPION did not extravasate to a noticeable degree in rats subjected to 1-h tMCAO and 1-day reperfusion, as shown in S4 Fig.

Comparisons of DSC-MRI times curves obtained following Dotarem vs. SPION injections

The experimental DSC-MRI-derived $\Delta R_2^*$ (t) curves for rats in the normal and tMCAO groups are shown in Fig 1A and 1C, respectively. The black arrows in the SPION time curves in Fig 1C indicate the apparent second peak due to recirculation [6,21]. This peak was usually unobservable in the Dotarem time curves. After the normalization of $AUC_{\text{Dotarem}}$ to $AUC_{\text{SPION}}$, $rCBV_{\text{Dotarem}}$ (nAUC$_{\text{Dotarem}}$) was significantly smaller than $rCBV_{\text{SPION}}$ (AUC$_{\text{SPION}}$) for the

![Fig 1. The process of nAUC ratio value estimation.](https://doi.org/10.1371/journal.pone.0201076.g001)
ipsilesional hemisphere in the tMCAO model (Fig 1D). rCBV_{error} was thus greater than 1 for the BBB-disrupted model. No significant differences in rCBV_{Dotarem} were observed between the two hemispheres in rats in the normal group (Fig 1B). rCBV_{error} was thus close to 1 for rats in the normal group, which did not have blood vessels with BBB disruption. In rats in the normal group, the AUC ratios were 0.613 and 0.596 for the right and left hemispheres, respectively. The corresponding rCBV_{error} values were 1 (by definition) and 0.971 for the right and left hemispheres, respectively. In rats subjected to tMCAO, the AUC ratios were 0.598 and 0.823 for the contralateral and ipsilateral hemispheres, respectively. This indicates post-ischemic hyper-perfusion caused by the SPION injection in the ipsilateral (ischemic) brain hemisphere [1]. The same hyper-perfusion was not observed following the Dotarem injection, presumably due to CBV and CBF underestimations associated with BBB leakage. The corresponding rCBV_{error} values were 1 (by definition) and 1.376 for the right and left hemispheres, respectively. The rCBV_{error} values for the six rats in the stroke group in this study are summarized in Table 1.

Quantified DSC-derived perfusion errors for normal and post-ischemic rat brains

Fig 2 shows the rCBV results for the Dotarem-Dotarem and Dotarem-SPION injections in normal rat brains, respectively. Fig 2A and 2B show the rCBV^{1st}_{Dotarem} (first injection) and rCBV^{2nd}_{Dotarem} (second injection) maps, which are used as controls, respectively. No noticeable differences were observed between the maps. Fig 2C displays the scatterplot of rCBV^{1st}_{Dotarem} values against rCBV^{2nd}_{Dotarem} values in the left hemisphere. Fig 2D and 2E show the rCBV_{Dotarem} (first injection) and rCBV_{SPION} (second injection) maps, respectively. No noticeable difference was observed between these maps, either. Finally, Fig 2F presents the scatterplot of rCBV_{Dotarem} values against rCBV_{SPION} values in the left hemisphere. The absence of significant differences (rCBV_{error} values were close to 1 for rats in the normal group) between rCBV^{1st}_{Dotarem} and rCBV^{2nd}_{Dotarem} and rCBV_{Dotarem} and rCBV_{SPION} confirm the consistency of the rCBV measurements obtained following successive CA injections of Dotarem and SPION (criterion standard) in non-leaking vasculature (normal condition) [22]. Fig 2G summarizes the statistical analysis results for each group. There were no significant differences (Wilcoxon rank sum test, p > 0.05, p = 0.136).

Fig 3 shows plots of cerebral perfusion measurement errors, such as rCBV_{error}, rCBF_{error}, and rMTT_{error}, for cases of post-ischemic local hypo- and hyper-perfusion. As seen in the ADC maps (Fig 3A2 and 3E2), the decreased ADC values and hyperintense $T_2$-weighted images (Fig 3A1 and 3E1) of the left hemisphere indicate the presence of ischemic stroke lesions after tMCAO reperfusion. Fig 3A3 and 3E3 illustrate the differences in the $T_1$ maps before and after the Dotarem injection. The significant differences in $T_1$ values in the ipsilateral hemisphere provide a clear indication of Dotarem leakage. No differences in $T_1$ values were observed following the SPION injections. Fig 3B1 and 3F1 and Fig 3B2 and 3F2, show the rCBV_{SPION} and the rCBF_{Dotarem} maps, respectively, while Fig 3B3 and 3F3 show the resulting rCBV_{error} due to CA extravasation. Fig 3C1 and 3G1, and Fig 3C2 and 3G2, show the rCBF_{SPION} and the rCBF_{Dotarem} maps, respectively, while Fig 3C3 and 3G3 show the resulting rCBF_{error}. Fig 3D1 and 3H1 and Fig 3D2 and 3H2, show the rMTT_{SPION} and rMTT_{Dotarem} maps, respectively, while Fig 3D3 and 3H3 show the resulting rMTT_{error}. In the case of hyper-perfusion, both rCBF_{SPION} and rCBF_{Dotarem} indicate the presence of post-ischemic ipsilateral hypo-perfusion with respect to the contralateral hemisphere, as indicated by the white arrow in Fig 3C2. In contrast, in the case of hyper-perfusion, rCBF_{SPION} indicates the presence of significant post-ischemic hyper-perfusion, while rCBF_{Dotarem} has non-significant differences with
Table 1. Mean and standard variations of ADC and rCBV error values for infarction, peri-infarction, and normal areas for six rats shown in Figs 4, 5 and 6.

| Rat | ADC (×10^6 μm²/s) | rCBV error (a.u.) | ADC (×10^6 μm²/s) | rCBV error (a.u.) | ADC (×10^6 μm²/s) | rCBV error (a.u.) | ADC (×10^6 μm²/s) | rCBV error (a.u.) |
|-----|--------------------|-------------------|--------------------|-------------------|--------------------|-------------------|--------------------|-------------------|
|     | Normal tissue      |                   | Normal tissue      |                   | Normal tissue      |                   | Normal tissue      |                   |
|     | 715 ± 105          | 1.063 ± 0.088     | 1.015 ± 0.270      | 672 ± 85          | 1.006 ± 0.109      | 1.153 ± 0.339      | 698 ± 86           | 0.991 ± 0.088      |
|     | Peri-infarct       | 645 ± 96          | 1.321 ± 0.189      | 641 ± 77          | 1.318 ± 0.224      | 1.388 ± 0.470      | 674 ± 59           | 1.274 ± 0.119      |
|     | Infarct            | 461 ± 21          | 1.207 ± 0.283      | -                 | -                 | -                 | -                  | -                 |

*p < 0.01 denotes Wilcoxon rank-sum test results for ADC values between normal and peri-infarct zones.

^ (p < 0.01) denotes Wilcoxon rank-sum test results for rCBV values between peri-infarct and infarct zones. a.u. means arbitrary unit.

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respect to the contralateral hemisphere, as indicated by the white arrow in Fig 3G2. These results clearly indicate that the DSC-MRI-derived CBV and CBF values were significantly underestimated when using gadolinium chelates, and in this case, led to errors in the detection of post-ischemic hyper-perfusion. In the ipsi-stroke hemisphere in both cases, the corresponding rCBV error and rCBF error were apparent not only in the infarcted zone (ADC < 500 μm²/s), but also in the peri-infarcted area. The boundaries of the region with significant rCBV error and rCBF error values mostly matched those of the corresponding T₁ difference map, indicating the leakage of Dotarem. The BBB leakage-derived errors did not appear to correlate with the Vₚ(rCBV SPION) values. It was also noted that when compared to the values obtained following the SPION injection, the rCBV Dotarem and rCBF Dotarem values were generally underestimated, and the rMTT Dotarem value was slightly overestimated. Fig 3I shows differences in the slopes of the scatterplots for the three perfusion indices obtained following the use of Dotarem and SPION in the BBB-disrupted region. The ROIs for these scatterplots were obtained from the corresponding T₁ difference maps. Rats in the normal group were used as controls. The perfusion parameters calculated in these rats were compared to those calculated in rats in the stroke group (Fig 3J). The rCBV error values for lesions in BBB-disrupted regions differed significantly from those obtained in rats in the control group (Wilcoxon rank sum test, p < 0.01, p = 1.98 × 10⁻⁷). In other words, the rCBV Dotarem values for lesions in the BBB-disrupted regions were estimated to be significantly lower (~20%) than the corresponding rCBV SPION values.

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Regional segmentation of post-ischemic brain based on rCBV_error, ADC, and $V_p$

In the first column of Fig 4, the infarct (ADC < 500 μm$^2$/s) voxels of regions with BBB damage (rCBV_error > threshold) are shown in red. The infarct (ADC < 500 μm$^2$/s) voxels of regions without BBB damage (rCBV_error < threshold) are shown in yellow, but are scarce. Voxels of
BBB-damaged regions (rCBV_{error} > threshold) without infarction (ADC > 500 μm^2/s) are shown in green. The green area may thus represent the peri-infarct area where there is BBB damage. In the second column of Fig 4, the ADC histograms for the infarct (ADC < 500 μm^2/s), peri-infarct with BBB damage (ADC > 500 μm^2/s and rCBV_{error} > threshold), and normal (ADC > 500 μm^2/s and rCBV_{error} < threshold) regions are shown in red, green, and purple, respectively. The overlapping ADC values between the peri-infarct (green) and normal (purple) areas are shown in dark blue. For the small lesions shown in the top two rows, the peri-infarct areas had noticeably smaller ADC values than areas of normal tissue. For the larger lesions shown in the bottom three rows, the presence of non-overlapping mid-range ADC values (500 μm^2/s < ADC < 600 μm^2/s) was distinct in the peri-infarct areas when compared to areas of normal tissue (indicated by black arrows on the ADC histograms). In the third column
of Fig 4, rCBV\textsubscript{error} histograms for the infarct, peri-infarct with BBB damage, and normal regions are shown in red, green, and purple, respectively. The overlapping rCBV\textsubscript{error} values between the infarct (red) and peri-infarct (green) areas are shown in dark orange. No significant differences were observed in the rCBV\textsubscript{error} values between the infarct (red) and peri-infarct (green) areas, as most of the red-tagged values overlap with green-tagged areas.

Table 1 summarizes the ADC, rCBV\textsubscript{error}, and rCBV\textsubscript{SPION} (V\textsubscript{p}) values for each region for the six rats subjected to stroke. The ADC values were significantly different for the infarct, peri-infarct, and normal regions. The rCBV\textsubscript{error} values were significantly different between the infarct (peri-infarct) and normal regions, but no difference was observed between the infarct and peri-infarct regions. The rCBV\textsubscript{SPION} (V\textsubscript{p}) values did not appear to strongly correlate with the applied regional segmentation.

Leakage compensation with CA pre-load

We performed DCE-MRI acquisitions before the DSC-MRI measurements with both Dotarem and SPION in three post-ischemic rats (a \(T_2\)-weighted image and an ADC map for a representative animal are shown in Fig 5A1 and 5A2, respectively). \(R_1\) (= \(1/T_1\)) changes are shown for infarcted (green) and normal (blue) regions as a function of post-injection time, where the injection time-points are marked with red lines on the axis in Fig 5A3. No significant \(T_1\) differences (after 10 min) were observed pre- vs. post-injection of Dotarem (0.1 mmol-kg\(^{-1}\)) in the infarction and normal regions, as shown in Fig 5A4. Similarly, no significant differences in vessel wall permeability (\(K_{trans}\)) values obtained using the extended Toft model were observed in the infarction region, as shown in Fig 5A5. After the Dotarem flush (0.2 mmol-kg\(^{-1}\) after 15 min), the \(T_1\) values were further reduced in the infarction region, but not in the normal region. This led to a significant difference in \(T_1\) (after 85 min) pre- vs. post-injection, as shown in Fig 5A3.

Perfusion deficits identified using fast DSC-MRI following increasing CA pre-loads are shown in Fig 6. Significant underestimation of rCBV\textsubscript{Dotarem} with respect to rCBV\textsubscript{SPION} without...
CA pre-load was again apparent, as shown in Fig 6A1, 6A2 and 6A3. However, in the presence of significant CA pre-load (net: 0.3 mmol kg\(^{-1}\)), the SPION and Dotarem rCBV maps were similar and there was minimal rCBV\(_\text{error}\), as shown in Fig 6C1, 6C2 and 6C3. In another rat with a comparable \(T_1\) difference map and CA pre-load (net: 0.3 mmol kg\(^{-1}\)), we repeatedly observed significantly reduced rCBV\(_\text{error}\), as shown in Fig 6D1, 6D2 and 6D3. In contrast, in a rat with a less conspicuous \(T_1\) difference map (Fig 6B5), a noticeable underestimation of rCBV\(_\text{Dotarem}\) was still observed, as shown in Fig 6B1, 6B2 and 6B3. Fig 6A4, 6B4, 6C4 and 6D4 compare scatterplots of rCBV values for the core region following Dotarem and SPION injections. Mismatches between the core and normal areas are reduced with increasing CA pre-load, as shown in the enlargement of the \(T_1\) difference map (post 85 mins) with increasing CA pre-load. No \(T_1\) difference map was available for no CA pre-load case and not available.

Discussion and conclusions

Significant perfusion deficits (rCBV\(_\text{error}\) ~20%) were present in DSC-MRI data obtained using fast (0.3 s) EPI acquisition, even in brains of post-ischemic rats (1-h MCA0, 24-h reperfusion) with insignificant permeability (\(K^\text{trans}\)) values obtained using DCE-MRI with gadolinium chelates administered at the conventional dose of 0.1 mmol kg\(^{-1}\). Even though it is difficult to separate the effects of \(T_1\) shortening and susceptibility contrast changes from those of leaking CA on DSC-MRI-derived perfusion deficits, fast EPI acquisition will be inevitably affected by \(T_1\) shortening due to the leaky vessels. Nevertheless, in animal model studies with rapid blood...
circulation at high magnetic fields, the lengthening of temporal resolution or the increasing of injection dose may not be adequate for the accurate determination of perfusion parameters. As a result, leakage compensation using CA pre-load (0.3 mmol·kg$^{-1}$) may be necessary to avoid perfusion deficits during high temporal resolution DSC-MRI acquisition in animal models of early post-ischemic reperfusion. This is especially true for longitudinal follow-up studies of post-ischemic animal models with varying degrees of vessel wall permeability [14].

It is also worthwhile to note that the DSC signal bias originates from compromised BBB integrity and is likely to be proportional to $K_{trans}$ and inversely proportional to CBV. As a result, the perfusion errors in DSC-MRI data obtained using extravasating vs. intravascular CAs may be particularly sensitive to weakly leaking microvessels with small CBVs. The conspicuous rCBV$_{error}$ in this tMCAO model may thus provide more sensitive diagnostic information than the corresponding ADC and rCBV$_{SPION} (V_p)$ maps. The increased area of the elevated rCBV$_{error}$ values for enlarging infarct regions may articulate the boundaries of the BBB-disrupted areas in the ipsilesional hemisphere in this ischemic reperfusion stroke model. The significantly larger regions with disrupted BBB than the diffusion-reduced (infarction) regions in the tMCAO model indicate peri-infarct BBB-damaged capillaries (Fig 3), which may lead to secondary vascular dysfunction that can limit recovery of viable tissue near an infarcted zone. A few histology-based studies have also confirmed the existence of BBB-damaged capillaries in peri-infarct zones [23,24]. In the peri-infarct BBB-damaged zone determined based on the rCBV$_{error}$ threshold, a significant reduction in the ADC value was apparent between 500 $\mu$m$^2$/s and 600 $\mu$m$^2$/s (Fig 3 and Table 1). The region with slightly decreased ADC values co-localized with the elevated rCBV$_{error}$, and is likely to be a signature of early vasogenic edema, some of which may progress to irreversible infarction.

The potential limitations of this study are as follows. First, direct histological comparison was not possible for the proposed rCBV$_{error}$ values, as independent three-dimensional characterization methods for vessel permeability and flow are scarce. Instead, considering the similar molecular weight of Dotarem (0.56 kDa) to that of traditional Evans blue dye (0.9 kDa), the $T_1$ difference maps obtained before and after the Dotarem injection (0.3 mmol·kg$^{-1}$) were used to assess BBB leakage in this study. Second, any Dotarem remaining in the tissue may impose a signal bias for the following DSC-MRI performed after the SPION injection. However, as the $\Delta R_2^*(t)$ of the early-phase DSC-MRI was obtained based on the signal differences before vs. after the CA administration rather than from the absolute signals, we assumed that the effects of remaining CA on the second injection were minimal. Future developments in fast $T_1$ acquisition methods with improved sensitivity [25,26] or the complete separation of $T_2^*$ and $T_1$ changes in the first-passage signal time courses for the leaky BBB may provide further insights into the biophysical mechanisms underlying multiple CA injections. Third, although the $\Delta R_2^*(t)$ values obtained at 7 T are minimally related to the underlying vessel sizes or shapes [27] and are primarily dependent on CBV and CBF values for randomly oriented vessels, cautious interpretation of $T_2^*$-based DSC-MRI signals is required due to potential geometric complications and unwanted susceptibility artifacts in post-ischemic brain applications. Future investigation of the effects of unaccounted Dotarem-cell interactions or vessel size effects on dual DSC-MRIs may be pursued in post-ischemic animal models of weak BBB damage.

In summary, we combined two different AUC measurements of sequential DSC-MRIs to characterize cerebral perfusion errors using extravasating (Dotarem) and intravascular (SPION) CAs in the brains of a post-ischemic 60-minute MCAO and 1-day reperfusion rat model. DSC-MRI-derived perfusion indices, such as relative CBF and CBV values obtained using an extravasating CA, were underestimated (~20%). The brain area with a significant rCBV$_{error}$ encompassed the region of infarct tissue and mostly co-localized with the region with $T_1$ differences pre- vs. post-Dotarem injection. This indicates the presence of a disrupted
BBB in the infarct and peri-infarct regions. The DSC measurements obtained using significant pre-load (0.3 mmol·kg\(^{-1}\)) of Dotarem had minimal perfusion deficits when compared to those obtained using the reference intravascular SPION.

**Supporting information**

**S1 Fig.** (a-1) Dynamic \(\Delta R_2^*\) curve from SPION in normal rat brain. (a-2) The ROI of the brain location, where the \(\Delta R_2^*\) curve (a-1) is sampled from. Time intervals of first passage (pre-injection~4s), second passage (4~10s) and steady state (>10s) were defined based on DSC-MR signal with intravascular SPION injection. (b) The ipsilateral (red) and contralateral (green) ROI of the brain locations, where the \(\Delta R_2^*\) curves of stroke rats were sampled for Fig 1C. (TIF)

**S2 Fig.** **Experimental schemes of animal preparation and MR scan.** (a) Experimental scheme for normal rats. (b) Experimental scheme for stroke group rats. A \(T_2^*\)-weighted image, ADC map, and \(T_1\) map were additionally obtained for the stroke group rats. The durations for \(T_2^*\)-weighted image (RARE), ADC map (DW-EPI), \(T_1\) map (RAREVTR), and AUC map (DSC-EPI) acquisitions were 10, 9, 10, and 4 mins, respectively. The duration between the injections for each experiment was 2 hours. The duration for MCAO and following reperfusion was 1 hour and 24 hours, respectively. (TIF)

**S3 Fig.** The process of nAUC estimation. (a) and (b) \(\text{AUC}_{\text{DOTAREM}}\) and \(\text{AUC}_{\text{SPION}}\) map from normal rat brain. (c) Scatter plot between \(\text{AUC}_{\text{DOTAREM}}\) and \(\text{AUC}_{\text{SPION}}\) for reference and interest region. (d) \(n\text{AUC}_{\text{DOTAREM}}\) map, which was divided by the ratio of \(\text{AUC}_{\text{SPION}}\) and \(\text{AUC}_{\text{DOTAREM}}\) (= 0.573). (e) Scatter plot between \(n\text{AUC}_{\text{DOTAREM}}\) and \(\text{AUC}_{\text{SPION}}\) for reference and interest region. (TIF)

**S4 Fig.** (a) The \(T_1\) difference before and after the CA injection for 1-hr MCAO and 1-day reperfusion model. The green and yellow bar graphs present the \(T_1\) difference of the ipsilateral infarction (ADC < 500 \(\mu\)m\(^2\)/s) and contralateral regions, respectively. The statistical unit \(n_{sl}\) is the number of slices. For the DOTAREM case (left), green bar: 252 ± 94 ms (\(n_{sl} = 12\)), yellow bar: 87 ± 39 ms (\(n_{sl} = 12\)), and \(p < 0.01\) (\(p = 0.001\)). For the SPION case (right), green bar: 27 ± 31 ms (\(n_{sl} = 12\)), yellow bar: 47 ± 26 ms (\(n_{sl} = 12\)), and \(p > 0.05\) (\(p = 0.229\)). (b) The \(T_1\) difference maps for 1-hr MCAO/1-day and 1-hr MCAO/7-day reperfusion models, respectively. Significant leakage of SPION is apparent in 1-hr MCAO/7-day reperfusion model. (TIF)

**S5 Fig.** Respective rCBV histograms of normal, peri-infarction, and infarction regions from six stroke rats, which were reported in Table 1. (TIF)

**S6 Fig.** The fittings of DCE-MRI time curves, which were used to generate \(K_{tr}V_a\) and \(V_p\) maps shown in Fig 5. Blue and red dots represent time-signal data for normal and infarction regions, respectively. (TIF)

**S7 Fig.** The fittings of ADC values, which were used to generate ADC maps shown in Figs 3–5. Blue and red dots represent diffusion data for normal and infarction regions, respectively for six stroke rats. (TIF)
S8 Fig. The fittings of rCBV values from DSC-MRI, which were used to generate rCBV maps throughout the manuscript.

(TIF)

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