RESEARCH ARTICLE

Model-free leakage index estimation of the blood-brain barrier using dual dynamic susceptibility contrast MRI acquisition

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Pharmacokinetic K_2 mapping from dynamic susceptibility contrast (DSC)-MRI can be a sensitive technique for evaluating the vascular permeability of the subtly damaged blood-brain barrier (BBB) in ischemic regions. However, the K_2 values of ischemic lesions depend upon the selection of the intact BBB reference region. As previous observations suggest that the ΔR_2^* curve of pre-loaded DSC-MRI is not significantly affected by the extravasation of contrast agent, dual DSC-MRI acquisitions can be performed to derive the BBB leakage index from the voxel-wise reference input function for ischemic regions. This study aims to demonstrate the robustness of such model-free leakage index estimation in ischemic brains. By configuring the relationship between dual ΔR_2^* curves of the intact contralateral brain, the deviation of the measured ΔR_2^* curve from the unloaded DSC-MRI with respect to the non-deviated ΔR_2^* curve in the pre-loaded DSC-MRI can be quantified as the BBB leakage index. Such model-free leakage index values from rats with transient middle carotid artery occlusion (tMCAO) ($n = 17$) and normal controls ($n = 3$) were evaluated and compared with conventional K_2 values with multiple reference regions. Inter-subject leakage index values were also compared with the corresponding ΔT_1 map. Evans-blue-stained images were used to validate the leakage index. For the tMCAO group, leakage index values correlated well with ΔT_1 (Pearson's $r = 0.828$). The hyperintense area on the leakage index map matched well with the corresponding Evans-blue-stained area (Dice correlation = 0.626). The slopes of the scatter-plot from the leakage index (0.97-1.00) were observed to be more robust against changes in the reference region than those from conventional K_2 values (0.94-1.07). In a subtly damaged BBB tMCAO model, model-free evaluation of vascular permeability using dual DSC-MRIs would provide a consistent measure of inter-subject vascular permeability.

KEYWORDS

DSC-MRI, DCE-MRI, Vascular permeability, Ischemic stroke, Blood Brain Barrier

Abbreviations: ADC, apparent diffusion coefficient; BBB, blood-brain barrier; CA, contrast agent; CBF, cerebral blood flow; DCE, dynamic contrast enhanced; DSC-MRI, dynamic susceptibility contrast MRI; EPI, echo planar imaging; FA, flip angle; FOV, field of view; NA, number of averages; NR, number of repetitions; NS, number of slices; RARE, rapid acquisition with relaxation enhancement; RAREVTR, RARE with variable T_R ; SD, Sprague-Dawley; SG, slice gap; SNR, signal-to-noise ratio; ST, slice thickness; T_E , echo time; tMCAO, transient middle carotid artery occlusion; T_R , repetition time.

1 | INTRODUCTION

In ischemic stroke, the disruption of the blood-brain barrier (BBB) is known to significantly contribute to subsequent neurological impairments.^{1–3} BBB changes in ischemia/reperfusion are dynamic and complex, and thus it is expected that dynamical and quantitative monitoring of BBB integrity could potentially lead to safer and improved outcomes with therapeutic measures, such as thrombolysis.^{4,5} Cerebral MRI for vascular permeability measurement is an essential imaging biomarker to evaluate the integrity of the BBB.^{6–8} For example, dynamic contrast-enhanced (DCE)-MRI measures the signal increase from Gd-based MR contrast agent (CA) injection and estimates the pharmacokinetic model-based transfer constant (K^{trans}). Although this K^{trans} reflects the mixture of vascular permeability and flow, for a low permeability condition like the early stage of ischemic stroke the K^{trans} may mainly represent vessel permeability.^{9,10} DCE-MRI relies upon T_1 relaxation time shortening due to leakage of the CA into interstitial spaces. As it requires a certain amount of CA accumulation for T_1 values to change, its sensitivity may be limited in detecting subtle BBB damage, as in the early stage of ischemic stroke, within short DCE-MRI acquisition time (<10 min).¹¹

Dynamic susceptibility contrast (DSC)-MRI is a T_2^* -based or T_2 -based technique that is typically used for evaluating cerebral blood volume, cerebral blood flow (CBF), and mean transit time in capillaries.¹² DSC-MRI has a basic assumption that the CA is not extravasated from blood vessels,^{13–15} but numerous studies have used the K_1 , K_2 model to study the effects of CA extravasation on the DSC-MRI signal in brains with BBB damage, especially for tumors and strokes.^{8,16,17} Specifically, to derive corresponding K_1 and K_2 values of lesions with a BBB disruption, the ΔR_2^* curve of the intact contralateral BBB region should be pre-determined as a reference input function. However, the averaged ΔR_2^* curve of the intact BBB reference region may not represent heterogeneous ΔR_2^* curves in the contralateral (normal) brain, which has various ΔR_2^* curve patterns depending on local anatomy. Therefore, the K_2 values provide model-dependent relative values depending on the selection of the intact BBB “reference region.” To compensate for such problems, the shape of the ΔR_2^* curve from the reference region is often adjusted by an additional time scaling factor and time offset.¹⁷ However, the introduction of a time scaling factor and offset cannot fully describe the shape of the ΔR_2^* curve from the reference region, and K_2 is still a model-derived value depending on the selection of the reference region. Therefore, if the voxel-wise reference input function, ie the non-deviated ΔR_2^* curve for each voxel of the ischemic lesion with BBB leakage, can be estimated, the dependence of DSC-MRI variable values on the choice of the reference region will be minimized to potentially provide a model-free BBB leakage index.

To directly measure the non-deviated ΔR_2^* curve of the regions with a disrupted BBB, pre-loaded DSC-MRI acquisition is a potential candidate method.^{18–21} As the extravasating CAs would cause T_1 reduction in tissue and consequently positively enhance the DSC-MRI signal, several previous studies have shown that extravasated CAs from the unloaded DSC-MRI (the first DSC-MRI) prevent such positive signal enhancement due to T_1 reduction in lesions with a damaged BBB during the pre-loaded DSC-MRI (the second DSC-MRI). No significant cerebral blood volume differences in the blood pool CA when using DSC-MRI and pre-loaded DSC-MRI were subsequently observed.^{18,19} Such observations suggest that the ΔR_2^* curve of pre-loaded DSC-MRI is not significantly affected by the extravasation of the CA. Therefore, by performing two sequential DSC-MRIs, the ΔR_2^* curve of the pre-loaded DSC-MRI could be considered a voxel-wise reference input function for the ΔR_2^* curve of the unloaded DSC-MRI in ischemic regions. By configuring the relationship between the unloaded and pre-loaded ΔR_2^* curves of the intact contralateral brain, the deviation of the measured ΔR_2^* curve from the unloaded DSC-MRI with respect to the non-deviated ΔR_2^* curve from the pre-loaded DSC-MRI can then be quantified as a BBB leakage index.

In this study, we propose a method to estimate vascular permeability that is robust to the reference region without pharmacokinetic modeling by using sequentially implemented dual DSC-MRI acquisitions. To verify the proposed method, preclinical rodent studies were performed step by step. First, the feasibility of the proposed method was confirmed in normal control rats. Second, to demonstrate the robustness of the leakage index against the choice of reference areas, the K_2 and leakage index based on multiple reference regions were directly compared in 1 h MCAO with 1 d, 3 d, or 5 d reperfusion rats. Finally, to address the accuracy in estimating the extension and the level of BBB leakage of the proposed method in ischemic lesions, the regions with disrupted BBB on the leakage index map were mutually validated with corresponding histological Evans blue staining and ΔT_1 maps, and inter-subject variations of leakage index values were compared with corresponding ΔT_1 values.

2 | MATERIALS AND METHODS

2.1 | Animal preparation

All experiments were approved by the institutional animal care and use committee. For the animal experiment, 7-week-old female Sprague-Dawley (SD) rats from Orient Bio (Gyeonggi, Republic of Korea) were used. The experimental group ($n = 17$) and control group ($n = 3$) consisted of ischemic stroke surgery rats and normal rats, respectively. The transient middle carotid artery occlusion (tMCAO) group underwent 1 h MCAO surgery with intraluminal monofilament (0.35 mm diameter filament, Doccol, Sharon, MA) with 1 d ($n = 6$), 3 d ($n = 6$), and 5 d ($n = 5$) of reperfusion depending on their group. During the 7 T MRI (Bruker BioSpin, Ettlingen, Germany) scan, rats were anesthetized with 1–2% isoflurane. The body temperature of rats was maintained at 37 °C by circulating warm water. Sequential dual DSC-MRI was performed to evaluate vascular permeability with a same injection dose and pulse sequence. Besides, to confirm the edematous area and region with damaged BBB, the apparent

diffusion coefficient (ADC) and longitudinal T_1 difference (ΔT_1) maps were acquired. The ΔT_1 maps were calculated by subtracting two different T_1 maps. After MRI scanning, a rat in the 3 d reperfusion group was used for Evans blue staining. Also, for DCE-MRI, 0.3 mmol/kg of Gd-DOTA was injected into one rat in the 1 d reperfusion model.

2.2 | MRI protocols

T_2 -weighted MR images were obtained using rapid acquisition with relaxation enhancement (RARE) with the following parameters: repetition time (T_R) = 5000 ms, RARE factor = 4, effective echo time (T_E) = 30 ms, number of averages (NA) = 2, field of view (FOV) = 30×30 mm², matrix size = 256×256 , number of slices (NS) = 3, slice gap (SG) = 0.2 mm, and slice thickness (ST) = 1 mm.

ADC maps were acquired using diffusion-weighted echo planar imaging (EPI) with the following parameters: T_R = 5000 ms; number of segments = 4; effective T_E = 20 ms; b values = 200, 400, 600, and 1000 s/mm²; NA = 1; FOV = 30×30 mm²; matrix size = 96×96 ; NS = 3; SG = 0.2 mm; and ST = 1 mm. Three ADC maps in the x , y , and z planes were averaged to obtain the mean ADC values.

T_1 maps were obtained using RARE with variable T_R (RAREVTR) with the following parameters: T_R = 80, 150, 200, 400, 800, 1200, 1600, 2000, 2500, 3000, and 4500 ms; RARE factor = 4; effective T_E = 5.9 ms; NA = 1; FOV = 30×30 mm²; matrix size = 96×96 ; NS = 3; SG = 0.2 mm; and ST = 1 mm. The total acquisition time of RAREVTR was 10 min. T_1 maps were acquired before and 5, 15, and 30 min after flushing of the injection line with saline. The flushing was performed by injecting saline as much as the dead volume of injection line (90 μ L) after unloaded DSC-MRI.

DSC-MRI perfusion maps were acquired using a gradient-echo EPI sequence with shortened repetition time to capture the T_1 effect of extravasating CA. The detailed pulse sequence parameters are as follows: T_R = 300 ms, effective T_E = 17 ms, NA = 1, FOV = 30×30 mm², matrix size = 96×96 , NS = 3, SG = 0.2 mm, ST = 1 mm, bandwidth = 3.5×10^5 Hz, number of segments = 1, flip angle (FA) = 30° , number of repetitions (NR) = 800, and temporal resolution = 0.3 s. Baseline and total acquisition times for DSC-MRI were 1 min (200 images) and 4 min, respectively. For the dual DSC-MRIs, two DSC-MRI experiments were performed sequentially. For the unloaded DSC-MRI (the first DSC-MRI), 0.2 mmol/kg of Gd-DOTA was injected. After the first injection, the remaining Gd-DOTA (~ 0.1 mmol/kg) in the dead volume (90 μ L) of the injection line was flushed with 90 μ L of saline. The pre-loaded DSC-MRI (the second DSC-MRI) was performed 40 min (with three T_1 measurements between dual DSC-MRIs) after the flushing of the injection line. The injection dose and pulse parameters of the pre-loaded DSC-MRI were identical to the unloaded DSC-MRI.

DCE-MRI data were acquired using fast low-angle shot (FLASH) with the following parameters: T_R = 35 ms, T_E = 1.9 ms, NA = 1, FOV = 30×30 mm², matrix size = 96×96 , NS = 3, SG = 0.2 mm, ST = 1 mm, FA = 30° , NR = 180, and temporal resolution = 3.36 s. The dose of Gd-DOTA was 0.3 mmol/kg. Baseline and total acquisition times for DCE-MRI were 1 min (18 images) and 9 min, respectively. DCE-MRI was performed with one rat from the 1 d reperfusion group without additional DSC-MRI experiment.

2.3 | Ex vivo assessment of BBB breakdown and extent of ischemia

To confirm the region of BBB disruption, one rat from the 3 d reperfusion group was stained with Evans blue.²² While the rat was anesthetized, 2 mL/kg of Evans blue solution (4%) was injected via the tail vein. The brains of rats were perfused with saline 30 min after the injection and fixed with 4% PFA for 2 d. The brains were embedded in OCT compound and axially sliced in 100 μ m thick sections using a cryostat (Leica). After the sections were mounted and coverslipped, fluorescence images were acquired using a ZEISS Axio Zoom.V16 microscope with 63 ZEISS filter (excitation/emission wavelength = 572/629 nm). The spatial resolution of the acquired image was 1 μ m $\times$ 1 μ m.

2.4 | Data analysis

To confirm the edematous area, the ADC map in each direction (x , y , z) was calculated using a mono-exponential function.²³ The three ADC maps were averaged to obtain the mean ADC values.

To evaluate vascular permeability, K_2 and K^{trans} were computed from DSC-MRI and DCE-MRI, respectively. The DSC-MRI signal was converted to the ΔR_2^* curve ($= -1/T_E \ln[S(t)/S_{pre}]$, where $S(t)$ is the DSC-MRI signal and S_{pre} is an averaged signal from the pre-injection of DSC-MRI. To obtain K_2 values, each voxel of the ΔR_2^* curve was fitted using the following equation¹⁷:

$$\Delta R_2^*(t) = K_1 \Delta R_2^* \left(\frac{t + \tau}{\alpha} \right) - K_2 \int_0^t \Delta R_2^* \left(\frac{t' + \tau}{\alpha} \right) dt' \quad (1)$$

FIGURE 1 The processes of estimating the non-deviated unloaded DSC-MRI curve from the pre-loaded DSC-MRI curve by using a conversion matrix. A, The reference region (green) on the EPI image of the unloaded DSC-MRI and pre-loaded DSC-MRI, respectively. B, The ΔR_2^* curves of DSC-MRI are rearranged to t (the number of time points) $\times$ n (the number of voxels in reference region) matrices. The conversion matrix is computed using SVD deconvolution. C, The non-deviated ΔR_2^* curve of the unloaded DSC-MRI is estimated by multiplying the computed conversion matrix by the ΔR_2^* curve of the pre-loaded DSC-MRI. D, The estimated ΔR_2^* curve of the unloaded DSC-MRI and corresponding measured ΔR_2^* curve of the unloaded DSC-MRI from the right hemisphere in a normal rat

where $\overline{\Delta R_2^*}(t)$ represents the averaged ΔR_2^* curve for brain tissue regions with intact BBB, α is a time scaling factor, and τ is the arrival time. $\overline{\Delta R_2^*}(t)$ was computed by averaging the ΔR_2^* curves from the contralateral hemisphere.

To estimate the proposed BBB leakage index from dual DSC-MRI, the following procedures were performed, as schematized in Figure 1. For the intact BBB region, the ΔR_2^* curve is only affected by CA passage inside the vasculature embedded in the tissue of interest. Therefore, ΔR_2^* curves from DSC-MRI can be described in the form of a discretized matrix as follows²⁴: $\Delta R_2^* = C_A \cdot r_2^* \cdot \text{CBF} \cdot R$, where r_2^* is the transverse relaxivity of the CA, R is the residue function, and C_A is the matrix form of the arterial input function (AIF). Therefore, two sequential DSC-MRIs can be described as follows:

$$\begin{cases} \Delta R_{2,1st}^* = C_A^{1st} \cdot r_2^* \cdot \text{CBF} \cdot R \\ \Delta R_{2,2nd}^* = C_A^{2nd} \cdot r_2^* \cdot \text{CBF} \cdot R \end{cases} \quad (2)$$

where $\Delta R_{2,1st}^*$ and $\Delta R_{2,2nd}^*$ are the measured ΔR_2^* curves for the first- (unloaded) and second-injected (pre-loaded) DSC-MRI acquisitions, respectively. C_A^{1st} and C_A^{2nd} are the AIFs for the corresponding DSC-MRIs, respectively.

In Equation 2, the residue function describes the fraction of injected CA remaining in the vasculature. Also, Equation 2 makes the assumption that the residue function is time invariant.²⁵ Thus, if the same CA passes through the same tissue, the residue function is expected to be identical. Therefore, by assuming that the CBF and residue function (R) do not significantly change for the unloaded and pre-loaded DSC-MRI acquisitions, the equations can be rearranged as follows:

$$\Delta R_{2,1st}^* = C_A^{1st} \cdot C_A^{-1,2nd} \cdot \Delta R_{2,2nd}^* \quad (3)$$

where $C_A^{-1,2nd}$ is the inversion matrix of C_A^{2nd} . In Equation 3, $C_A^{1st} \cdot C_A^{-1,2nd}$ describes the relationship between the unloaded and pre-loaded DSC-MRIs. Therefore, if the $C_A^{1st} \cdot C_A^{-1,2nd}$ matrix is known, $\Delta R_{2,2nd}^*$ can be consistently converted to $\Delta R_{2,1st}^*$. To calculate the conversion matrix ($C_A^{1st} \cdot C_A^{-1,2nd}$), ΔR_2^* curves from the left hemisphere (contralateral hemisphere in the stroke group) can be considered an intact BBB region that satisfies Equation 3, as shown in Figure 1A and 1B.

$$\begin{bmatrix} \Delta R_{2,1st}^*(1,1) & \cdots & \Delta R_{2,1st}^*(1,n) \\ \vdots & \ddots & \vdots \\ \Delta R_{2,1st}^*(t,1) & \cdots & \Delta R_{2,1st}^*(t,n) \end{bmatrix} = C_A^{1st} \cdot C_A^{-1,2nd} \begin{bmatrix} \Delta R_{2,2nd}^*(1,1) & \cdots & \Delta R_{2,2nd}^*(1,n) \\ \vdots & \ddots & \vdots \\ \Delta R_{2,2nd}^*(t,1) & \cdots & \Delta R_{2,2nd}^*(t,n) \end{bmatrix}. \quad (4)$$

Next, the conversion matrix was calculated by multiplying the ΔR_2^* data matrix from the unloaded DSC-MRI by the inversion matrix for the ΔR_2^* data matrix from the pre-loaded DSC-MRI. The inversion matrix was computed based on singular value decomposition (SVD) with a threshold (10^{-5} , this value was empirically set) of the maximum singular value.²⁶ The estimation of ΔR_2^* curves from the pre-loaded DSC-MRI was processed using a conversion matrix, as shown in Figure 1C.

If CA is extravasated to the tissue, the estimated ΔR_2^* curves will be different from the measured ΔR_2^* curves. Therefore, the difference reflects the level of BBB damage and can be defined by the following equation:

$$\text{leakage index} = \int_0^t (\text{estimated } \Delta R_2^*(t') - \text{measured } \Delta R_2^*(t')) / r_2^* dt'. \quad (5)$$

After the derivation of proposed leakage index values following the above procedures, to compare the similarity between the K_2 map and leakage index map, the lesions with BBB leakage were defined as those that satisfied fixed criteria ($\Delta T_1 > 0$, $K_2 > 10^{-4}$, and leakage index > 0), and a scatter-plot between K_2 and leakage index values from the legion with damaged BBB was plotted. To further study the effects of the choice of intact BBB regions (reference region) on the K_2 or leakage index maps, four different ROIs (contralateral hemisphere, contralateral cortex, contralateral striatum, and contralateral corpus callosum) were used in deriving K_2 and leakage index values. To offset any constant biases in the values of K_2 and leakage index within the contralateral hemisphere, all K_2 and leakage index values had with the averaged K_2 and leakage index values of the contralateral hemisphere subtracted.

To study the accuracy of the proposed method in estimating the extension of BBB leakage lesions, the correlation between the leakage map and two different ΔT_1 maps (ΔT_1 map between pre-injection and 5 min after flushing the injection line ($\Delta T_{1,0min-5min}$ map), ΔT_1 map between 5 min and 30 min after flushing the injection line ($\Delta T_{1,5min-30min}$ map)) were mutually compared. Here, the ΔT_1 map reflects the amount of CAs passed through the disrupted BBB during a specific period. The hyperintense areas in the leakage index map were overlapped with the hyperintense areas in the $\Delta T_{1,0min-5min}$ or $\Delta T_{1,5min-30min}$ maps. To show the accuracy in estimating the degree of BBB leakage, the corresponding mean values of the leakage index and $\Delta T_{1,0min-5min}$ from the lesion with BBB damage were scatter-plotted. For the histological validation of the proposed method, the overlap between the leakage index map and Evans-blue-stained region was also directly compared.

To study the effects of the acquisition time of the leakage index map, five different time intervals (from 30 s to 165 s after CA injection) of ΔR_2^* curves were used for the leakage index map. To further study the robustness of the estimated leakage index against the arrival time gap between the unloaded and pre-loaded DSC-MRI, leakage index maps with intentionally varied arrival times (time difference = 15 s) were compared with matched ones. For the analysis of DCE-MRI acquisition, K^{trans} values were evaluated using the extended Tofts model.²⁷ The averaged DCE-MRI signal of the internal carotid artery (6 voxels) was considered as an AIF.

3 | RESULTS

To compare the sensitivities of DSC-based permeability (K_2) mapping and DCE-based permeability (K^{trans}) in characterizing subtly damaged BBB in ischemic lesions, Figure 2 shows the DCE-MRI and DSC-MRI results for a representative 1D reperfusion model. Hypointense areas on the ADC map and hyperintense areas on the ΔT_1 map (T_1 difference between pre-injection and 9 min after the CA injection for DCE-MRI or unloaded DSC-MRI) show the existence of edema and the location of BBB damage, respectively. There is a clear contrast between the contralateral hemisphere and the BBB damaged region on the K_2 map, but such contrast is not apparent on the corresponding K^{trans} map. Figure 2C and 2D shows the averaged raw ΔR_1 (from DCE) and ΔR_2^* (from DSC) curve for the region with BBB damage, respectively. Signal enhancement due to CA extravasation T_1 -shortening effects (black ellipses in Figures 2C and 2D) start to appear only after about 250 s (after injection) for DCE-MRI acquired ΔR_1 , but such enhancement was observed about 50 s after injection for DSC-MRI acquired ΔR_2^* curves.

To show the efficacy of the proposed method for BBB-damaged ischemic brains, representative voxel-wise ΔR_2^* curves of model-free (conversion matrix approach) leakage index estimation were shown for normal and ischemic brains on different reperfusion days. As a result of the dual DSC-MRI acquisitions, the estimated ΔR_2^* curve from the pre-loaded DSC-MRI acquisition and the ΔR_2^* curve from the unloaded DSC-MRI were compared in normal rats and tMCAO rats, as shown in Figure 3. Figure 3A shows the quality of estimation for a single voxel ΔR_2^* curve from a normal rat. Each ΔR_2^* curve comes from a red arrowed voxel on the leakage index map (Figure 3A, middle). The estimated ΔR_2^* curves from the left hemisphere (Figure 3A, left) and right hemisphere (Figure 3A, right) have high r -squared values (0.9730 and 0.9648, respectively) for the normal brain with intact BBB. As shown in Figure 3B, there was no significant difference in r -squared values between the left and right hemispheres in the three normal rats ($p = 0.109$, paired t -test with 95% confidence level). However, as shown in Figure 3C and 3D, there were clear differences between measured (red dots, from the unloaded DSC-MRI) and estimated ΔR_2^* curves (blue line, from the pre-loaded

FIGURE 2 Comparison between DCE-MRI and DSC-MRI in 1 h MCAO 1 d reperfusion model. A, B, The ADC map, ΔT_1 map, and corresponding permeability map, respectively. Both ΔT_1 maps were acquired 9 min after CA injection and the total CA dose was the same (0.3 mmol/kg). C, D, The averaged relaxation rate curves (~ 110 voxels were averaged) in ipsilateral and contralateral regions in DCE-MRI and DSC-MRI, respectively. The time at which the relaxation rate between the ipsilateral and contralateral regions begins to differ is indicated by a black ellipse, and is significantly shorter for ΔR_2^* than ΔR_1^*

FIGURE 3 Representative voxel-wise ΔR_2^* curves of model-free (conversion matrix approach) leakage index estimation for normal and ischemic brains. A, C, D, The leakage index map, estimated ΔR_2^* curve for the unloaded DSC-MRI, and corresponding measured ΔR_2^* curve of the unloaded DSC-MRI for normal, 3 d, and 5 d reperfusion model rats, respectively. Each blue line and solid red dot show the estimated and measured ΔR_2^* curve in a single voxel as indicated by the red arrow. B, Bar graphs of the r -squared value for the left and right hemispheres. The height and error bar represent the mean value and standard deviation of r -squared values for three normal rats. There is no significant difference between the left and right hemispheres ($p = 0.109$, paired t -test)

DSC-MRI) for the 3 d and 5 d reperfusion models, due to BBB leakage in ischemic lesions. The degree of deviation appeared to be larger for the 5 d reperfusion model. Each representative ΔR_2^* curve was obtained from one voxel indicated by the red arrow in each leakage index map (Figure 3C and 3D).

FIGURE 4 Comparison between K_2 and leakage index maps. A-D, Representative T_2 -weighted, ADC, K_2 , and leakage index maps for a 3 d reperfusion model, respectively. E, Scatter-plot correlations between K_2 and leakage index values in a region with damaged BBB for the corresponding maps

FIGURE 5 The representative K_2 and leakage index map with the choice of different reference regions. B-E, K_2 or leakage index maps with the corpus callosum or striatum as the reference region. A, The corresponding regions are marked in blue and green, respectively

To address this similarity between the conventional K_2 map and the proposed leakage index map, Figure 4 shows the visual comparisons and associated correlation scatter-plot for a representative model. Figure 4C and 4D shows the K_2 and leakage index maps for the 3 d reperfusion model, respectively. The T_2 -weighted image and ADC map of the corresponding slice are shown in Figure 4A and 4B, respectively. The K_2 and leakage index values in the regions with BBB damage show good mutual correlation (Pearson's $r = 0.8625$), as shown in Figure 4E. The mean $\pm$ standard deviation of Pearson's r for 16 rats were 0.649 ± 0.158 .

To demonstrate the repeatability of the proposed method against the different choices of reference ROI selection, Figure 5 shows the representative mapping results comparing the ROI dependences of the reference region for K_2 and the leakage index map. Figure 5B and 5C shows K_2 and leakage index maps that were computed by considering the contralateral corpus callosum as a reference region (the reference region was used to calculate the conversion matrix and $\Delta R_2^*(t)$ for the leakage index map and K_2 map, respectively.). The K_2 (Figure 5D) and leakage index maps (Figure 5E) were calculated using the contralateral striatum as the reference region. Reference regions for the corpus callosum and striatum in the contralateral brain are shown in blue and green in Figure 5A. A noticeable bias was observed for the K_2 map from the choice of different ROIs of the reference region, while consistent leakage index maps were obtained regardless of the variation of reference ROIs.

To further validate the robustness of the leakage index map against reference region variations, Figure 6 shows the ROI dependence of the K_2 and leakage index map with all experimental ischemic brains on varying reperfusion days ($n = 16$). Selected reference regions for the cortex (red), corpus callosum (blue), striatum (green), and entire contralateral hemisphere (red, blue, green, and yellow) are shown in Figure 6A. The red area in Figure 6B corresponds to the representative ROI for the scatter-plots (Figure 6C and 6D). Figure 6C and 6D shows multiple scatter-plots between three different reference regions against the entire contralateral region for K_2 and leakage index in the region with BBB damage, respectively. The slopes of the scatter-plot for K_2 (0.94-1.07, 14% error) were consistently lower than those for plots of the leakage index (0.97-1.00, 3% error), which shows the robustness of the leakage index estimated from dual DSC-MRI acquisitions.

Next, to verify the accuracy in estimating the extension and the degree of BBB-leakage by the proposed leakage index mapping, the overlap and correlation between the leakage index and ΔT_1 maps are shown in Figure 7. Figure 7A shows the detailed MRI scan procedure

FIGURE 6 Variations of K_2 and leakage index values with various reference regions. C, D, Correlation scatter-plot for K_2 and leakage index values, respectively, between the values derived from various regions (cortex, striatum, and corpus callosum) with respect to the values derived from the contralateral region. The red, green, and blue colors in each scatter-plot indicate the K_2 or leakage index values chosen with the cortex, striatum, or corpus callosum as the reference region; the corresponding region is marked in the same color in A. A, The area in the contralateral hemisphere, which includes the red, green, blue, and yellow areas. Only the K_2 or leakage index values in the region with BBB damage are used for the scatter-plot; the region with BBB damage is defined based on the same criteria ($\Delta T_1 > 0$, $K_2 > 10^{-4}$, leakage index > 0). B, The corresponding region is shown in red

FIGURE 7 The correlation between ΔT_1 and leakage index map for the extension and the level of BBB leakage. A, The MRI scan scheme for the tMCAO group. B–D, The representative leakage index map, ΔT_1 between 5 min after and 30 min after flushing the injection line ($\Delta T_{1,5min-30min}$), and ΔT_1 between pre-injection and 5 min after the flush ($\Delta T_{1,0min-5min}$) for a 5 d reperfusion rat model, respectively. E, F, The similarity of threshold masks between the leakage index map and $\Delta T_{1,5min-30min}$, or between the leakage index map and $\Delta T_{1,0min-5min}$, respectively. The red, green, and yellow-colored areas represent the threshold mask from the ΔT_1 map ($\Delta T_{1,5min-30min} > 180$ ms and $\Delta T_{1,0min-5min} > 725$ ms), the leakage index map (> 50 a.u.), and the area where the two masks overlap, respectively. G, The correlation scatter-plot between the mean value of the leakage index and $\Delta T_{1,0min-5min}$ in the region with BBB damage in 16 rats. Two points (one point is 1 d reperfusion [183, 21.6] and the other is 3 d reperfusion [184, 21.6]) overlap in G

for the tMCAO group. Correspondingly, Figure 7B–D shows the leakage index map, ΔT_1 map between 5 min and 30 min ($\Delta T_{1,5min-30min}$ map), and ΔT_1 map between pre-injection and 5 min ($\Delta T_{1,0min-5min}$ map) for 5 d reperfusion rats, respectively. Figure 7E and 7F shows hyperintense regions of the leakage map (green area in E and F), $\Delta T_{1,5min-30min}$ map (red area in E), and $\Delta T_{1,0min-5min}$ map (red area in F). The region of the overlap region between the leakage index and ΔT_1 map is shown as a yellow area in Figure 7E and 7F. The hyperintense regions of the leakage index map were observed to be surrounded by the $\Delta T_{1,5min-30min}$ map, as shown in Figure 7E (Dice correlation = 0.1301). However, the leakage index map and $\Delta T_{1,0min-5min}$ map show similar contrasts, as shown in Figure 7F (Dice correlation = 0.6897). Figure 7G shows a significant correlation between the average leakage index and $\Delta T_{1,0min-5min}$ values for 16 rats (Pearson's $r = 0.8279$).

Finally, for the histological validation of the proposed method, the overlap between the leakage index map and Evans blue-stained region is shown in Figure 8. Figure 8A–C shows the leakage index map, T_2 -weighted image, and fluorescence image for the 1 d reperfusion model. The hyperintense regions of the leakage index map and fluorescence image correspond to the red and green areas in Figure 8D, respectively. The masks corresponded well, and the Dice correlation was 0.6263. The observed overlaps and correlations of leakage index with respect to histology and $\Delta T_{1,0min-5min}$ map respectively validate the extension and the degree of BBB leakage, which are estimated from the dual DSC-MRI acquisition-derived leakage index.

Additionally, the effects of scan-time durations of the DSC-MRI on the leakage index map are shown in Supporting Information Figure S1. The extent of the leakage index map was observed to decrease with shorter scan-time duration. In particular, for the 105 s scan-time duration (Supporting Information Figure S1c), vascular permeability contrast at the bottom of the BBB damaged region was no longer detected. The effects of the arrival time gap between the unloaded and pre-loaded DSC-MRIs on the leakage index are shown in Supporting Information Figure S2. Supporting Information Figure S2a and S2c shows the quality of estimation of one voxel's ΔR_2^* curves in normal tissue and ischemic lesions for 5 d reperfusion rats. The ΔR_2^* curves in Supporting Information Figure S2a and S2c are from the voxels indicated by red arrows in Supporting Information Figure S2b. Supporting Information Figure S2d and S2f shows the quality of estimation of ΔR_2^* curves, which have a 15 s arrival time difference between the unloaded and pre-loaded DSC-MRIs and show the same results in Supporting Information Figure S2a and S2c. Supporting

FIGURE 8 Comparison between the leakage index map and Evans-blue-stained image. A-C, Leakage index map, T_2 -weighted image, and fluorescence image for the 3 d reperfusion model. D, Similarity between the leakage index map and Evans-blue-stained image. The red, green, and yellow areas represent the threshold mask from the Evans-blue-stained image, the leakage index map, and the area where the two masks overlap, respectively

Information Figure S2b and S2e shows similar vascular permeability contrast regardless of the arbitrarily imposed potential arrival time variabilities between the two DSC-MRIs.

4 | DISCUSSION

Although DCE-MRI is widely used for evaluating vascular permeability, previous studies have shown that DCE-MRI has limited sensitivity for evaluating subtle ischemic vascular permeability.^{11,28–30} A long DCE-MRI acquisition time (~ 20 min) would enhance the contrast as a result of the increased amount of extravasated CA.^{31–33} However, extravascular extracellular space volume (v_e) and CA diffusion begin to affect the amount of extravasated CA over time and may not directly represent spatial vascular permeability.³⁴ Therefore, to evaluate subtle vascular permeability, it will be more appropriate to minimize the duration of dynamic acquisition, while achieving a sufficiently large MR contrast to distinguish the BBB leakage lesion due to direct CA extravasations. In this study, the ΔR_2^* curve based on DSC-MRI showed significant signal deviation due to CA leakage with a minimized duration (~ 100 s) of dynamic acquisition, which was apparently much shorter than the required duration for corresponding DCE-MRI acquisition. This is also probably because the longer T_R and the larger T_2^* effects of DSC-MRI than DCE-MRI produce a higher signal-to-noise ratio (SNR) and contrast-to-noise ratio. Apart from the benefit for the SNR, decreasing the susceptibility difference between the tissue and vasculature with extravasating CA may further reduce ΔR_2^* values of the unloaded DSC-MRI, in addition to ΔR_1 effects.

Several studies have consistently shown that pre-loaded DSC-MRI acquisition provides a ΔR_2^* curve with minimized CA extravasations. By using the conversion matrix obtained by comparing ΔR_2^* curves between sequential DSC-MRI acquisitions of regions with intact BBB, the non-extravasated ΔR_2^* curve of a single voxel can be extracted from the pre-loaded DSC-MRI for lesions with BBB leakage. As each voxel's ΔR_2^* curves of unloaded and pre-loaded DSC-MRI originate from the same vascular structural information, the associated leakage index should be

independent of blood volume, dispersion, or arrival time. Therefore, leakage index values appear to avoid the modulation problem faced by conventional K_2 model fits without the need to introduce a time scaling factor or time offset. In addition, the leakage index relies only on the relationship between the unloaded and pre-loaded DSC-MRI, with no need for pharmacokinetic modeling. Therefore, the leakage index may also be less error-prone due to discrepancies between the pharmacokinetic model and measured data.

The limitations of this study are as follows. First, the change in physiological state between the unloaded and pre-loaded DSC-MRI may alter CBF in Equation , which would potentially cause an error in the conversion matrix estimation. If changes occur throughout the brain, the effects on the conversion matrix are not significant. However, if there is a local CBF change in the lesion after the unloaded DSC-MRI, the conversion matrix could be incorrectly computed. Such a potential error can be further reduced by reducing the time interval between the unloaded and pre-loaded DSC-MRIs. By shortening the time interval between the unloaded and pre-loaded DSC-MRIs as much as possible, the local CBF changes between the unloaded and pre-loaded DSC-MRIs may be further minimized and a more accurate conversion matrix can be provided. Second, the effects of the remaining CA in blood on the leakage index have not been explored. To increase the accuracy of the conversion matrix, the interval between the unloaded and pre-loaded DSC-MRIs should be reduced as much as possible. However, if there is not sufficient time between the unloaded and pre-loaded DSC-MRI, the remaining CA from the unloaded DSC-MRI may shift the baseline DSC-MRI curves of the pre-loaded DSC-MRI. Therefore, to determine the optimal time interval, the effects of remaining CA in blood should be systematically studied. To achieve this, the error of leakage index may be evaluated by shortening the time interval between the unloaded and pre-loaded DSC-MRIs. If the effects of the time interval on the accuracy of leakage index are studied, the dual DSC-MRI method can be readily applied to patients because the concept of pre-loaded DSC-MRI is already well established in the clinic. Third, from a practical point of view, the dose of CA is limited. However, half the dose (0.05 mmol/kg) used in typical clinical DSC-MRI scans can be used for each injection in clinical dual DSC-MRIs studies. When calculating the conversion matrix, the closer the dual DSC-MRI conditions, the more accurate the conversion matrix would be. However, considering the linear response of ΔR_2^* with different doses,²⁷ the contrast may be improved by optimizing injection doses between dual DSC-MRI acquisitions.

In summary, the feasibility of estimating a non-extravasated ΔR_2^* curve in a lesion with BBB leakage based on the pre-loaded DSC-MRI was demonstrated. The corresponding leakage index was computed by evaluating the difference between the non-extravasated (estimated from the pre-loaded DSC-MRI) and extravasated (measured from the unloaded DSC-MRI) ΔR_2^* curves. Such leakage index maps showed similar contrasts to conventional K_2 maps but were observed to be more robust against the choice of reference region with intact BBB. The validity of the leakage index as a measure of the degree of inter-subject BBB integrity was validated with corresponding in vivo ΔT_1 values. The area of the elevated leakage index matched well with the corresponding histological Evans-blue-stained image. While dual CA injections are frequently performed for pre-loaded DSC-MRI in preclinical and clinical settings, the proposed model-free leakage index estimation technique from dual DSC-MRI may improve the robust quantification of inter-subject vascular permeability, especially for lesions with subtle BBB damage in ischemic brains.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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