Dynamic contrast-enhanced MRI and CT provide comparable measurement of blood–brain barrier permeability in a rodent stroke model

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A B S T R A C T

In the current management of acute ischemic stroke (AIS), clinical criteria are used to estimate the risk of hemorrhagic transformation (HT), which is a devastating early complication. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and computed tomography (DCE-CT) may serve as physiologically-based decision making tools to more reliably assess the risk of HT. Before these tools can be properly validated, the comparability of the blood–brain barrier (BBB) permeability measurements they generate should be assessed. Sixteen rats were subjected to a transient middle cerebral artery occlusion before successively undergoing DCE-CT and DCE-MRI at 24-hours. BBB permeability ($K_{trans}$) values were generated from both modalities. A correlation of $R = 0.677$ was found ($p < 0.01$) and the resulting relationship was $[DCE-CT] = (0.610 * DCE-MRI) + 4.140$. A variance components analysis found the intra-rat coefficient of variation to be 0.384 and 0.258 for $K_{trans}$ values from DCE-MRI and DCE-CT respectively. Permeability measures from DCE-CT were 22% higher than those from DCE-MRI. The results of this study demonstrate for the first time comparability between DCE-CT and DCE-MRI in the assessment of AIS. These results may provide a foundation for future clinical trials making combined use of these modalities.

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1. Introduction

Acute ischemic stroke (AIS) causes a loss of blood–brain barrier (BBB) integrity, which can exacerbate neuronal ischemia and lead to hemorrhagic transformation (HT) [1–3]. The only approved treatment for AIS is thrombolysis, which can itself further degenerate the BBB and has been shown to increase the risk of HT [4]. The use of thrombolysis for AIS therefore involves balancing the benefit of reperfusion against the risks of HT [5]. In order to restrict thrombolysis to AIS patients with a lower risk of HT, clinical criteria are employed, including a strict therapeutic window of 4.5 hours. However, advanced imaging techniques, such as dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and computed tomography (DCE-CT), may serve as physiologically-based decision making tools to improve the assessment of HT risk in patients with AIS [6].

Both DCE-MRI and DCE-CT have undergone validation testing against gold-standard histological techniques and are able to quantify BBB permeability in vivo [7–12]. After AIS, early increased BBB permeability within infarcted brain tissue has been shown to correlate with the eventual risk of developing HT [13]. A number of human studies have measured BBB permeability in AIS patients with DCE-MRI [6,13–15] and DCE-CT [16] and have predicted the eventual risk of HT with good sensitivity and specificity. There is potential that BBB permeability assessment may be able to improve clinical decision-making around AIS. However, despite its benefits, the clinical adoption of BBB permeability assessment faces challenges. One challenge is the differing use of imaging modalities between stroke treatment sites. Depending on site-specific protocols, stroke centres may use MRI, CT, or a combination of the two modalities during the work-up of AIS patients [17]. Thus, it is relevant to question whether BBB permeability measurements gathered from DCE-MRI and DCE-CT would be comparable.

To date no studies have directly measured the comparability of BBB permeability measurements gathered from DCE-MRI and DCE-CT after AIS within the same experiment. Such assessments are difficult and require the use of an animal model, as human experimentation would be associated with ethical concerns due to the time-sensitive nature of AIS management. In a rodent tumor experimentation would be associated with ethical concerns due to the time-sensitive nature of AIS management. In a rodent tumor model permeability measures from DCE-MRI and DCE-CT were found to have non-equivalent permeability estimates, but similar reproducibility [18]. The failure of this study to demonstrate equivalence between DCE-MRI and DCE-CT may be explained by the small sample size and the non-physiological nature of the tumor model.

To overcome these limitations, a stable rodent model of AIS was generated in which pilot studies demonstrated promising reproducibility between DCE-MRI and DCE-CT results [19]. Based on these findings, a randomized controlled study comparing DCE-MRI and DCE-CT was conducted in a rodent stroke model. In this study, the results from DCE-MRI and DCE-CT were compared for the first time using the same model and with a larger sample size. The aim of this study was to determine whether DCE-MRI and DCE-CT could be used interchangeably to estimate BBB permeability in a rodent stroke model.
its usage of different pharmacokinetic models for CT and MRI (Tofts and deconvolution physiological models). BBB permeability values from DCE-MRI and DCE-CT for AIS patients have been published separately but comparisons are difficult as these studies have used differing models and imaging parameters and no effort has been made to directly compare the modalities within a single experiment [16,19,20]. As a result there is currently no consensus regarding the agreement between DCE-MRI and DCE-CT.

In the present study we directly compared the measurement of BBB permeability obtained successively with DCE-MRI and DCE-CT in a rodent model of ischemic stroke with reperfusion. We hypothesized that BBB permeability measures from the two methods would be correlated if the same pharmacokinetic model was used.

2. Materials and methods

2.1. Experimental design

The experimental time-line is as shown in Fig. 1. Sixteen male Sprague-Dawley rats (Charles River; MA, USA) weighing between 250 and 300 g were used for this study. Rats underwent a transient middle cerebral artery occlusion (tMCAo) surgery to model AIS and reperfusion injury [21]. Twenty-four hours later rats underwent DCE-CT immediately followed by an MR scanning protocol that included DCE-MRI. Following the completion of scanning all rats were humanely sacrificed with an IP injection of 100 mg/kg of phenobarbital sodium (Animal Health Inc., Quebec). The local institutional animal care committee approved all procedures and protocols for this study in accordance with the Canadian Council on Animal Care requirements.

2.2. Transient middle cerebral artery occlusion

Rats were anesthetized (isoflurane 3% induction, 1–2% maintenance) and focal ischemia was induced with the tMCAo model. A commercially available silicone-coated filament measuring 0.35 mm × 5–6 mm (Doccol, Sharon, MA) was used to increase the reproducibility of the experimental stroke [22]. The silicone coated suture has been shown to result in more complete occlusion of the MCA. The middle cerebral artery (MCA) was occluded by the suture for 1 hour before the suture was carefully withdrawn to reperfuse the cerebral circulation without causing endothelial damage. Breathing rate and temperature were monitored and temperature was maintained at 36.1 °C. Rats were given 2 mL of saline subcutaneously immediately after and 5–hours after occlusion. Post-operative analgesia was provided with 0.03 mg/kg Buprenor-phine (RB Pharmaceuticals ltd., Berkshire).

2.3. Neurologic testing

To confirm success of the tMCAo, neurologic testing was performed at 1-hour and 24-hours post-occlusion. Neurologic testing consisted of the modified Garcia neurologic score [23]. Briefly, a composite score of spontaneous activity, limb symmetry, forepaw outstretching, climbing, body proprioception, and response to vibrissae touch was generated. Rats showing no neurologic deficit at 1-hour (neurologic score > 15) were excluded from the rest of the experiment.

2.4. Dynamic contrast-enhanced CT imaging

Rats were anesthetized (isoflurane 3% induction, 1–2% maintenance) for both CT and MRI scans. CT data were acquired on a pre-clinical CT scanner (Locus Ultra microCT; GE Healthcare; WI, USA). Dynamic CT images were obtained in 42 mm coronal sections parallel to the skull base with 0.46 mm slice thickness, 80 kV and 95 mA. In-plane image resolution was 0.15 × 0.15 mm² with a field of view of 10 cm. A 1.0 mL dose of iodinated contrast media (Visipaque, 300 mg/ml; GE Healthcare; WI, USA) was administered by intravenous bolus injection via the tail vein during the first 12 seconds of the scan. Images were acquired at 1 second intervals for the first 30 volumes, with the remaining 15 volumes spaced 10 seconds apart. The total scan time was 3 minutes. A water phantom was placed under the animal to aid in hysteresis correction during post-processing. After scan completion, all data were transferred to an independent workstation for further analysis.

2.5. Dynamic contrast-enhanced MR imaging

MRI was performed immediately after CT scanning using a 7.0 T, 30 cm NMR imaging spectrometer (Bruker Biospec 70/30; Bruker Bio Spin MRI GmbH; Ettlingen, Germany). In addition to DCE imaging, structural data were acquired to evaluate surgical outcome as well as the size and location of the infarct. To maintain consistency, all structural images were acquired with an FOV of 30 mm × 30 mm and an acquisition matrix of 200 × 200. A time-of-flight sequence (2D TOF, TR/TE = 21.5/4.5 ms, slice = 0.4 mm) was run to confirm reperfusion in the MCA. For all other structural scans, 8 coronal slices were acquired with a thickness of 1.0 mm. These scans included diffusion-weighted images (DWI, with b = 0.1000, TR/TE = 1000/33 ms), T1-weighted Q2 spin echo images (T1FLASH, TR/TE = 500/3 ms), and T2-weighted turbo-spin echo images (T2RARE, TR/TE = 4800/72 ms). Dynamic contrast-enhanced (DCE) imaging was acquired with an FOV = 32 × 32 × 8 mm, image acquisition matrix = 128 × 128 × 8, flip angle = 30°, and a temporal resolution = 7.25 seconds, TR = 4.83 ms, TE = 1.87 ms (where it is assumed TE << T2*) [24,25]. A tail vein bolus of gadolinium-DTPA (Gadovist, 2 mmol/ml; Bayer Schering; Berlin, Germany) was injected after the acquisition of the second dynamic of the DCE scan at a dose of 60 μl over 12 seconds. The total acquisition time was 5:08 minutes for a collection of 42 volumes.

2.6. Image analysis

DWI data were converted to apparent diffusion coefficient (ADC) maps using an in-house software package (MR analyst v2.1) developed in MATLAB (MathWorks; Natick, MA, USA). ADC maps were calculated by fitting the normalized logarithmic signal-intensity decay as a function of the b-values. In the ADC maps, areas of ischemia were identified as regions of reduced diffusion when compared to the normal cortex.

Two regions of interest (ROI) were defined: one placed within the core of the diffusion abnormality (infarct) and the second in the homologous region of the contralateral cerebral hemisphere (Fig. 2). Infarct volumes were calculated from the ADC maps by summing the area of the infarct ROIs across slices.

Using the MNI-register v1.4.0 tool (MINC, Montreal, QB, Canada), images between the two modalities were manually aligned to generate a corresponding transformation matrix for each rat. The matrix was applied to the CT image using mincresample v1.2.1 (MINC, Montreal, QB, Canada), thus registering the CT data to the

Fig. 1. The experimental time-line outlining the relative time points of surgical procedures and imaging.

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MRI orientation, spatial resolution and slice thickness. The accuracy of co-registration was verified by visual inspection.

DCE data for both MRI and CT were processed using the same tool (MR analyst) to calculate coefficients of BBB permeability estimates ($K_{\text{trans}}$) from image signal intensities [26]. Prior to DCE-CT analysis, however, hysteresis correction was performed by normalizing the signal of each volume relative to the mean signal fluctuations observed in the water phantom. For both DCE-CT and DCE-MR images, the signal from the sagittal sinus was sampled to generate a vascular input function of the contrast bolus. Next, the infarct and control ROIs, as defined on each slice of the ADC maps, were overlaid onto the DCE images. The DCE signal within each ROI was spatially averaged to represent the time-varying tissue concentration of the contrast agent. A unidirectional, two-compartment kinetic model was implemented to associate the computed vessel signal intensity and tissue signal intensity in the form of a Patlak plot. The Patlak model does not require a pre-contrast T1 map. In DCE imaging of the brain, the “permeability-limited” assumption can be applied and $K_{\text{trans}}$ is not influenced by blood flow, even when measuring regions of cerebral infarction. For each slice, a $K_{\text{trans}}$ value of the infarct and contralateral ROI was calculated from linear regression of their respective Patlak plot as previously described [26].

2.7. Statistical analysis

The correlation between the two modalities was analyzed with a linear random effects model with a random intercept per rat as previously described [27]. We did not utilize a random slope per rat. The $R^2$ value was calculated when both ignoring and accounting for clustering. In this way we compared $K_{\text{trans}}$ values from DCE-MRI and DCE-CT slice-by-slice while accounting for clustering within rats. The level of agreement between rats was visualized on a Bland–Altman plot. A variance component analysis was performed with the restricted maximum likelihood approach to estimate the intra- and inter-rat variance. All statistical analyses were completed in the SPSS software (IBM—2013, v.21).

3. Results

Three rats were excluded from the experiment because of insufficient stroke size or intra-cerebral hemorrhage after surgery leaving a final group size of 13 rats with 49 infarcted slices for comparison. Within this group the weight and physiologic parameters did not vary significantly. All rats exhibited contralateral neurologic deficits 24-hours after occlusion (neurologic score <15). All rats showed reperfusion of the ipsilateral MCA by 24-hours after occlusion. The average infarct size 24-hours after occlusion as measured on T2-weighted anatomic images was $0.231 \pm 0.092 \text{cm}^2$.

Representative permeability maps from DCE-MRI and DCE-CT are seen in (Fig. 2.) In both modalities the $K_{\text{trans}}$ was inhomogeneous throughout the infarct although this was more apparent in DCE-MRI. In general, DCE-MRI yielded lower $K_{\text{trans}}$ values within the infarct than did DCE-CT with a mean of 6.777 mL/kg-min and 8.275 mL/kg-min for DCE-MRI and DCE-CT respectively. The two modalities, however, generated similar $K_{\text{trans}}$ values within the non-infarcted tissue with a mean of 5.812 mL/kg-min and 5.429 mL/kg-min for DCE-MRI and DCE-CT respectively. The $K_{\text{trans}}$ within the non-infarcted tissue correlated significantly between DCE-MRI and DCE-CT by a linear mixed model, $R = 0.781$ ($p < 0.001$). The intra-rat coefficient of variation (CV) within the infarct was 0.258 and 0.384 for DCE-CT and DCE-MRI respectively. The inter-rat CV within the infarct was 0.453 and 0.512 for DCE-CT and DCE-MRI respectively (Table 1).

The linear mixed model showed a correlation of $R = 0.677$ ($p < 0.01$) between the DCE-MRI and DCE-CT when accounting for clustering within rats (Fig. 3). The resulting relationship was $\text{DCE-CT} = (0.610 \times \text{DCE-MRI}) + 4.140$. There was no evidence of heteroscedasticity in the Bland–Altman plot the discrepancies between the two modalities remained consistent with increasing $K_{\text{trans}}$ and no proportional bias error was evident (Fig. 4). The average difference between DCE-MRI and DCE-CT was $-1.498 \text{mL/kg-min}$ and the standard deviation of the differences was 2.947 mL/kg-min. The

![Fig. 2. Representative diffusion weighted images (DWI), and permeability maps generated from dynamic contrast-enhanced (DCE) MRI and CT. Blue coloring represents low permeability and red represents higher permeability.]
maximum difference between the modalities was $-6.695$ and $98\%$ of the differences fell within $2$ SD of the average.

## 4. Discussion

To our knowledge, this study is the first to present a direct comparison of BBB permeability measurements from DCE-MRI and DCE-CT in a rodent stroke model using the same pharmacokinetic model. DCE-MRI and DCE-CT scanning was conducted sequentially, allowing direct comparison of BBB permeability within the same lesion. Imaging protocols and contrast agents were chosen to replicate those used in clinical practice as much as possible. The intra-rat coefficient of variation of $K^{\text{trans}}_{\text{CT}}$ from DCE-CT was $0.258$. A previous rodent study reported a similar value of $0.23$ [18]. Studies in larger animals have also reported similar or higher values. In implanted rabbit tumors the CV was $0.24$ [28] and in dogs a value of $0.87$ [29] was reported. In our experiment DCE-MRI had an intra-rat CV of $0.384$. This value is similar to a previous study that reported a value of $0.21$ when assessing rat stroke lesions [20]. In addition, DCE-MRI studies on rat tumor models have reported similar values to ours [30].

The intra-rat CV was lower in DCE-CT than in DCE-MRI ($0.258$ vs. $0.384$). Our data acquisition with DCE-CT had a higher temporal resolution during the perfusion phase than the DCE-MRI, which may have resulted in a more robust measurement and lower CV. In addition, DCE-CT benefits from the direct relation between Hounsfield unit and iodine concentration, while DCE-MRI relies on native tissue T1, water exchange, and contrast agent relaxation. Regardless, the observed difference in variation between the two modalities was marginal and would likely not impact comparisons between BBB permeability measures gathered from DCE-MRI and DCE-CT.

We observed that DCE-MRI tended to give lower values for BBB permeability within infarcted tissue than DCE-CT. A previous study that compared permeability measures between DCE-MRI and DCE-CT in a rodent tumor model also found a systematic difference between permeability measures from the two modalities [18]. This finding may partially be a consequence of the shorter scan duration of DCE-CT imposed by the clinical scanner. Studies have noted that an abbreviated DCE protocol for both CT and MRI can overestimate permeability measures as the transfer of contrast between blood and tissue compartments may still be approaching equilibrium [15,31,32]. In addition, due to the influence of MRI contrast agent on CT density measures we were forced to always perform CT imaging before MRI. As a result, it is possible that BBB permeability may have been consistently affected by the prolonged anesthesia during the second (MRI) more than in the first (CT) portion of our study. In a rodent model of ischemic stroke isoflurane has been shown to lower blood pressure and attenuate BBB permeability in a time-dependent fashion [33]. Lastly, Visipaque has a larger molecular weight (1550 Da vs. 605 Da) and differing polarity when compared to Gadovist, which may affect its propensity to cross the BBB.

Nonetheless, the linear mixed model revealed a correlation of $R = 0.677$ between the two modalities and there was no evidence of heteroscedasticity. The line of best fit had a slope lower than the theoretical line of agreement, which means that a simple conversion factor may be necessary to bring the permeability measures from the two modalities into agreement with each other. Before such a conversion factor is proposed a better understanding of the influence of various parameters, such as contrast agent effects and prolonged isoflurane anesthesia should be achieved.

The Bland–Altman plot of permeability showed no discernible bias across the range of permeability measures. The average difference was below zero, reflecting once again the tendency for DCE-MRI to generate lower permeability values than DCE-CT. Only $29\%$ of observations had a difference greater than one standard deviation from the mean. Further investigation revealed that these observations tended to be of smaller infarcts, making it likely that

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Table 1: Summary of DCE-MRI and DCE-CT parameters with variance components analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DCE-MRI–$K^{\text{trans}}_{\text{MRI}}$</th>
<th>DCE-CT–$K^{\text{trans}}_{\text{CT}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units</td>
<td>mL/kg-min</td>
<td>mL/kg-min</td>
</tr>
<tr>
<td>Mean</td>
<td>6.777</td>
<td>8.275</td>
</tr>
<tr>
<td>SD</td>
<td>3.630</td>
<td>3.428</td>
</tr>
<tr>
<td>Range</td>
<td>1.402–16.240</td>
<td>2.081–15.960</td>
</tr>
<tr>
<td>Intra-rat CV</td>
<td>0.384</td>
<td>0.258</td>
</tr>
<tr>
<td>Intra-rat variance (%) of total variance</td>
<td>41%</td>
<td>38%</td>
</tr>
<tr>
<td>Inter-rat variance (%) of total variance</td>
<td>59%</td>
<td>62%</td>
</tr>
</tbody>
</table>

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the larger discrepancy in these observations was related to the partial volume effect near the periphery of the infarct [34]. This illustrates the importance of strategies for ROI selection that avoid infarct edge 'noise'. Methods to suppress partial volume effects have been developed and allow for accurate quantification of subtle BBB permeability disruptions [35]. Notwithstanding this, in our experiment, greater than 95% of the differences fell between two standard deviations of the mean, which fulfilled a generally accepted standard of repeatability between tests [36].

The correlation we identified between DCE-MRI and DCE-CT diverges from a previous analysis, which found no correlation in a rodent tumor model [18]. However, unlike this previous analysis, we used the same pharmacokinetic model and similar imaging parameters between DCE-MRI and DCE-CT. Our results demonstrate the importance of consistent modeling between modalities if comparable BBB permeability values are to be achieved. Due to scanner-specific constraints, temporal resolution differed between our DCE-MRI and DCE-CT scans. In DCE-MRI 7.25-second intervals were used throughout, but in DCE-CT 1 second intervals during the perfusion phase were followed by 10-second intervals during the permeability phase. It is possible that had the temporal resolutions between the two modalities been identical, a higher degree of correlation would have been found. The total length of image acquisition also differed between the two modalities (5 minutes for DCE-MRI and 3 minutes of DCE-CT). We have previously shown that the uncertainty in $K_{trans}$ decreases as the length of image acquisition increases [15]. However, only a slight improvement was found when 5 minutes of data was used as opposed to 3 minutes.

In our protocol ROIs were defined slice-by-slice on ADC maps derived from MRI and then subsequently applied to our dynamic CT images using a transformation matrix. This presents a limitation of our analysis, as it does not mimic a clinical scenario in which ROIs would be defined and dynamic scanning would be performed solely using CT. However, ischemic brain tissue can be reliably distinguished from ischemic penumbra and unaffected tissue in AIS patients by using CT perfusion imaging [37,38]. As a result, in the clinical setting DCE-CT could be combined with CT perfusion imaging to define the ROI. Another limitation of our study is that we manually defined the infarcted area during image analysis. Although a quantitative method would likely be used in the clinic, manual tracing is commonly used in pre-clinical studies and evidence suggests that accurate and precise definition of infarcted tissue is achieved regardless [39-41].

In the clinical setting AIS patients are most often first imaged with CT, but may subsequently undergo MRI during follow-up [17,42]. However, MRI is superior to CT to investigate more subtle strokes with minimal neurologic deficit [43]. The use of DCE imaging in the clinical management of AIS patients would require BBB permeability measures from MRI and CT to be interpreted in relation with each other. The results of the present study indicate that DCE-MRI and DCE-CT generate comparable measures of BBB permeability with similar variability and could perhaps be used interchangeably. Furthermore, although numerous trials have demonstrated the utility of BBB permeability assessment in AIS, these studies have been limited in their impact because of small samples sizes [14]. All such trials have used either DCE-MRI or DCE-CT alone. The results of the present study indicate that it may be possible to design a trial making use of both DCE-MRI and DCE-CT.

5. Conclusions

In conclusion, we have demonstrated a correlation between BBB permeability measures from DCE-MRI and DCE-CT after ischemia/reperfusion injury. This finding allows $K_{trans}$ values from the two modalities to be interpreted in relation with each other and perhaps used interchangeably. As a result, future human studies may consider incorporating both DCE-MRI and DCE-CT into their design to assess the robustness of these methods in larger patient populations.

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