Continuous Blood-Brain Barrier Breakdown In Acute Ischemic Stroke Patients

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Abstract

Neurological diseases such as ischemic stroke and dementia are associated with compromised blood-brain barrier (BBB) permeability. Knowledge about the time course of BBB leakage may have impact on therapeutic interventions and diagnostic measures such as testing for blood biomarkers. However, reports on the timeline and pattern of this leakage are contradictory. Therefore, we aimed to assess the time course of BBB permeability in ischemic stroke patients during the first 24 hours after symptom onset using dynamic contrast enhanced (DCE) MRI at 3 Tesla. We categorized time from stroke symptom onset to imaging into the following groups 1) 0-6 hours (n=10), 2) 6-16 hours (n=14) and 3) 16-24 hours (n=29). BBB permeability differed significantly between stroke lesions and the contralesional tissue for groups 2 and 3 ($p=0.006$, $p<0.001$, Wilcoxon-signed rank test). Using univariate or multivariate linear regression analyses we found no association between BBB leakage and age, sex, hyperintense reperfusion marker (another marker of BBB permeability) hemorrhagic transformation, white matter lesion load, symptom severity, functional disability and cerebrovascular risk factors. The results of our study therefore suggest continuous BBB leakage in the first 24 hours after stroke.

Introduction

The blood-brain barrier (BBB) acts as an interface between the vasculature and the brain tissue regulating the bi-directional passage of substances as well as protecting the central nervous system. The endothelial cells are the main physical barrier with the junctional complex of tight junctions, adherens junctions and GAP junctions. These are surrounded by pericytes, astrocytes and the basal membrane[1]. Neurological diseases such as ischemic stroke compromise the BBB function with increased permeability and potential consequences like brain edema formation, hemorrhagic transformation and polymorphonuclear neutrophil infiltration[2].

However, there are contradicting reports on the time course of the blood-brain barrier leakage after ischemic stroke and its association with reperfusion. Data from experimental animal models have suggested a biphasic[3,4] or even triphasic pattern[5] of BBB leakage. Huang et al.[3] reported a very early peak between 1.5-2 minutes and a late BBB opening occurring between 4 and 22 hours after ischemic injury. Klohs et al.[4] using near infrared spectroscopy and a radiolabeled albumin compound found an initial BBB opening at 4-8 hours and a second one at 12-18 hours after reperfusion. Preston et al.[5] using [^{3}H]sucrose also found the first opening 10 minutes after ischemia induction, followed by a second one after 6 hours in certain brain structures, such as striatum and hippocampus and a third opening between 6 – 24 hours in the neocortex. These regional differences suggest that different brain areas may have different sensitivity to ischemia and reperfusion. Applying various models, tracers or contrast agents injected impede comparison between studies.

On the other hand, there are also reports of a more continuous opening of the BBB after ischemic stroke in animal models as well as in humans[2,6,7]. The earliest time point of BBB leakage is reported to occur

within minutes after stroke onset and the leakage lasts for about 5 weeks in animal models[2]; in humans, however, the peak occurs between 6 to 48 hours after stroke onset[7].

Other factors besides ischemic stroke may influence blood-brain barrier permeability as well, for instance hyperglycemia[8] though, even here with contradicting reports[9]. Furthermore, when using MRI, factors such as main magnetic field strength, coil setups, sequence types, injection protocols as well as choice of arterial/venous input function can influence the assessment of BBB leakage as well as the quality of the generated parametric maps[10].

The aim of our study was to determine whether a time dependency of increased BBB permeability can be observed within the first 24 hours after stroke onset using dynamic contrast enhanced (DCE) MRI at 3 Tesla (T). We also assessed potential factors influencing BBB leakage.

Materials And Methods

Patients

Fifty-three patients of the ongoing prospective observational LOBI-BBB study were enrolled (clinicaltrails.gov NCT02077582). Inclusion criteria was time between stroke onset and imaging ≤ 24 hours. Exclusion criteria were age < 18 years, contraindications to MRI examinations such as pacemaker, strokes of unknown onset as well as renal dysfunction, since contrast agent was applied.

Written informed consent was obtained from all patients. The study design was approved by the local ethics committee of the Charité-Universitätsmedizin Berlin, Germany (EA4/056/08).

All methods were performed in accordance with relevant guidelines and regulations such as the Declaration of Helsinki and the “Strengthening the Reporting of Observational Studies in Epidemiology” (STROBE) guidelines for the reporting of observational studies.

Imaging

MR examinations were performed on a 3 T MRI scanner (Prisma_fit, Siemens Healthineers Erlangen, Germany) using a standard stroke protocol[22] as well as a T1 dynamic protocol. For T1 mapping T1 measurements with 4 different flip angles (2°, 10°, 20°, 35°) were acquired. Subsequently, a continuous serial acquisition of 60 volumes of T1-weighted images after administration of 10 mL gadobutrol (Gadovist®, 1 M, Bayer Schering Pharma AG, Berlin, Germany) at a flow rate of 1 mL/s and a delay of 1 minute, followed by a 20-mL saline flush covering a period of 6 minutes was performed. The imaging parameters for the T1 measurements were as follows: echo time (TE) 2.5 ms, repetition time (TR) 55 ms, 7 slices, 5 mm slice thickness, gap 0.5 mm, matrix size 128 x 102. TR for the different flip angle measurements was 60 ms.

Postprocessing of the data included motion correction[23] (FLIRT=FMRIB's linear image registration tool, FSL, FMRIB, Oxford, UK, (https://fsl.fmrib.ox.ac.uk/fsl/)) and coregistration of diffusion-weighted (DWI) and T1 dynamic images using SPM12 (Statistical Parametric Mapping, Wellcome Trust Centre for Human Neuroimaging, UCL, London, https://www.fil.ion.ucl.ac.uk/spm/). Regions of interests (ROI) of stroke lesions were created on coregistered DWI images and mirrored to the contralesional side (https://www.nitrc.org/projects/mricron/). Hemorrhagic transformed parts of ischemic stroke lesions are easily identified on DWI images and were excluded from the lesion ROI. Another ROI was placed in a major venous sinus and used as venous input function since the ΔR1 measured in the sagittal sinus agrees well with the time course of ΔR1 in arterial blood[24].

For BBB assessment the open source software package ROCKETSHIP (https://github.com/petmri/ROCKETSHIP) was employed using the Patlak model[10].

Statistics

Statistical analysis was performed using R (https://www.R-project.org/)[25]. To compare K^{trans} values between stroke and mirror regions the Wilcoxon signed-rank test was employed. Univariate analysis was first used to assess the association between log-transformed K^{trans} values and parameters like TSI, HARM[12] sign (the presence of contrast agent in the cerebrospinal fluid detected on FLAIR), hemorrhagic transformation, and cerebrovascular risk factors . The log transformation of the K^{trans} values was performed to deal with some violations of the assumptions of univariate and multivariate linear regression. A p-value of < 0.05 was considered as significant.

Results

Fifty-three patients were included in our study (mean age 73.7 (±10.3) years, 11/53 females (20.8%)). The patient sample was divided according to time between stroke onset to imaging (TSI): 1) 0-6 h (n=10, 18.9%), 2) 6-16 h (n=14, 26.4%) and 3) 16-24 h (n= 29, 54.7%)[11], with the mean time being 15.3 (±7.9) hours. Eighteen patients received iv tPA. In 21 patients (44.7%) HARM sign (=hyperintense reperfusion marker)[12] and, in 3 patients (6.4%) a hemorrhagic transformation of the infarct lesion was observed. In the majority of cases (n=35, 66.0%) the infarcts were located in supratentorial regions. Additional patient characteristics according to TSI categories are given in Table 1.

Table 1
Patient characteristics

	NIHSS d1	mRS d1	mRS d90	Wahlund	Infarct volume (mL)	n CRF
0-6 h (n=10)	1 (0-2)	2 (2-3)	2 (1-3)	5 (4-8)	0.67 (0.38-6.37)	2 (2-3)
6-16 h (n=14)	1 (1-3)	2 (1-3)	1 (1-3)	7 (2-12)	1.21 (0.35-3.94)	2 (2-3)
16-24 (n=29)	2 (1-4)	3 (1-4)	1 (0-2)	8 (2-12)	0.59 (0.22-1.76)	2 (2-3)

NIHSS=National Institutes of Health Stroke Scale Score, mRS=modified Rankin Scale, Wahlund=Wahlund score[13], CRF=cerebrovascular risk factor

Values are given as median and interquartile range (IQR)

For 0-6 hours no significant differences in K^{trans} values were found (p=0.123). Significant differences in K^{trans} values between stroke and mirror regions were observed for groups 2 and 3 (6-16 h (p=0.006) & 16-24 h (p<0.001)), see Table 2, figure 1.

Table 2
K^{trans} values according to TSI onset categories

TSI	K^{trans}, median, IQR		
	stroke ROI	**mirror ROI**	p*
0-6 h (n=10)	0.254 (0.072-0.723)	0.054 (0.0-0.460)	0.123
6-16 h (n=14)	0.230 (0.024-0.58)	0.025 (0.0-0.103)	0.006
16-24 (n=29)	0.304 (0.064-0.532)	0.055 (0.0-0.241)	< 0.0001

TSI=time between symptom onset and imaging, IQR=interquartile range, K^{trans} is given in 10^{-3} min^{-1}

p* = Wilcoxon sign rank test

In a first step a univariate linear regression analysis was performed to assess the association between TSI groups 2) and 3) respectively (p=0.983 and 0.622, R^2=0.008) and Wahlund score[13] (p=0.933, R^2=0.0) stroke location (p=0.096, R^2=0.053), HARM sign (p=0.776, R^2=0.002), iv-tPA (p=0.159, R^2=0.039), hemorrhagic transformation (p=0.590, R^2=0.007), stroke volume (p=0.643, R^2=0.004), age (p=0.240, R^2=0.004), sex (p=0.528, R^2=0.008), NIHSS day 1 (p=0.601, R^2=0.005), NIHSS day5 (p=0.894, R^2=0.0004), mRS day 1 (p=0.671, R^2=0.081), mRS day 5 (p=0.662, R^2=0.065) and infarct K^{trans} values.

There were no associations between cerebrovascular risk factors (chronic infarctions/TIA p=0.487, R^2=0.0095), smoking (p=0.530; R^2=0.008), atrial fibrillation (p=0.628, R^2=0.005), coronary heart disease (p=0.153, R^2=0.040), dyslipidemia (p=0.381, R^2=0.015), diabetes mellitus (p=0.270, R^2=0.024), hypertension (p=0.492, R^2=0.009) and K^{trans} values either.

Multilinear regression analysis also did not reveal any associations (R^2=0.132, p = 0.433) between K^{trans} values and TSI (6-16 h: p=0.867, 16-24 h: p=0,139), Wahlund score[13] (p=0.827), stroke location (p=0.099), HARM sign (p=0.434) and hemorrhagic transformation (p=0.298)

Discussion

In acute stroke patients within 24 hours after symptom onset, we found a significant leakage of the BBB starting at 6 hours after stroke onset. Cerebrovascular risk factors, white matter hyperintensities expressed as Wahlund score[13], age or sex showed no association with BBB permeability.

Continuous increase of BBB permeability was described by Strbian et al.[2] in rats as well as by Merali et al. in humans[7]. Strbian et al[2] reported the earliest BBB opening 25 minutes after 90 minutes of ischemia-reperfusion up until 5 weeks with 2, 4, 6, 12, 18, 24, 36, 48, 72 hours and 1, 2, 3, 4 weeks follow-up examinations in between. The authors employed two contrast agents: Gd-DTPA (MW 590 Da, Magnevist, Schering, German) for T1 measurements in-vivo and EBA post-mortem (fluorescent dye, Sigma, MW 75.8 kDa, Sigma-Aldrich, Steinheim, Germany). The most pronounced BBB leakage for Gd-DTPA was found between 48h up until 2 weeks after ischemia most pronounced at 6 and 72 hours as well as 2 weeks after reperfusion. For EBA the most pronounced BBB leakage was found at 6 hours as well as 36 - 48 hours after reperfusion followed by a slow decline until the third week. Interestingly, for both markers of BBB leakage the first peak was observed at 6 hours after ischemia despite their different molecular weights. We found a significant BBB leakage at 6-16 hours after stroke onset which coincide with Strbian et al´s[2] results in rats. However, we did not find a significant earlier BBB leakage which might be due to the different species, experimental settings with a 4.7 T MR scanner and a bird cage RF coil used in the study by Strbian et al.[2] in contrast to our study with a 3 T MR scanner and a 64 channel head coil. Higher field strength might have an influence on the detection of BBB leakage[10].

In one of our recent studies[14], we also found a more than 100% increase in BBB leakage between the first BBB assessment within 48 hours and the follow-up examination between day 5-7 after symptom onset, supporting the observation of Strbian et al.[2], indicating a continuous, however fluctuating increase in BBB permeability.

Merali et al.[7] also found a continuous increase in BBB permeability, again most pronounced between 6 to 48 hours after stroke onset, but also in the hyperacute phase, that is within the first 6 hours after ischemia. The authors though, used the parameter permeability-surface area product (PS) instead of K^{trans} , which only corresponds to K^{trans} under very specific conditions. Only if cerebral blood flow considerably exceeds PS does K^{trans} approximately equal PS[15]. Compared to Merali et al.[7] with 20 patients in the hyperacute phase we only had 10 patients and used a different software package for the Patlak analysis, which might contribute to the difference in the results. Furthermore, BBB leakage is not only restricted to the stroke region but was also found in contralesional brain tissue[14] in the acute phase of stroke which we found in 6/10 patients in the first group. This might also contribute to the lack of

significant difference in BBB permeability in the first 6 hours after symptom onset. When contralesional BBB leakage recovers, as has been reported[14], differences in BBB leakage between stroke and contralesional tissue become more pronounced.

As already pointed out, differences in equipment such as MR scanner field strength, coil configuration, duration of ischemia, tracers, experimental design, software packages and kinetic models impact the quantification of BBB leakage[2,10], in particular with respect to a probable second BBB opening.

When applying Patlak analysis, which is used in most of the studies, Manning et al.[16] found factors yielding erroneous results when assessing BBB permeability. These include the rate of injection of contrast agent (CA). This can result in fast BBB water exchange and thereby in a substantial bias of the permeability surface product and occurs during the early part of the time course of the contrast agent application. A slow injection is less sensitive to plasma flow, since the slower exchange in Gadolinium concentration results in similar arterial, capillary and venous concentrations. Excluding early data points resolves the problem of the fast BBB water exchange, reduces blood flow effects and the sensitivity to plasma flow. Furthermore, a small delay for the bolus injections has significant impact on the estimated parameters and significant errors remain even when early data points are excluded, while slow injection of Gadolinium virtually eliminates the problem. T1 saturation and transverse dephasing caused by the high concentration of a bolus injection can be avoided as well[16]. Since we employed a slow injection protocol and started the analysis using the very first time points of signal intensity increase we could address these factors mentioned above to minimize bias in our analysis.

Complicating the reported results is the relative lack of longitudinal studies of BBB leakage. One such study was performed by Durukan et al. in rats[6] at five time points after reperfusion (2, 24, 48, 72 hours and 1 week) and showed persistent BBB leakage during the whole week. Another longitudinal study by our group in acute stroke patients within 48 hours after stroke onset showed a continuous increase in BBB leakage at 24-hour follow-up[14]. Follow-up studies in humans are complicated by the repetitive application of Gadolinium with its well-known potential side effects of nephrogenic systemic fibrosis[17] and evidence of deposition in certain brain regions[18,19].

As previously reported[14], we found no association between K^{trans} values and HARM sign, another reported marker of BBB disruption, which was observed in 21 out of 53 of patients in this current study. This can be explained by a different pathophysiological mechanism resulting in HARM sign in contrast to an increased K^{trans}. Additional factors influencing HARM sign are infarct size (median volume size 0.73, IQR 0.35-1.89 mL), which showed a large variability in our study cohort, as well as risk factors such as hypertension and diabetes[20]. Both were reported to have an impact on HARM sign and showed no association with K^{trans} values in our study using DCE-MRI, again probably due to the two different techniques applied. We also did not find an association between K^{trans} and hemorrhagic transformation, but the number of patients was too small (n=3) for any satisfactory analysis and conclusion.

Our study has some limitations, foremost the small number of patients assigned to each time between symptom onset and imaging category. This was largely due to the fact that many patients who were eligible for mechanical thrombectomy did not receive a DCE-MRI scan, as this would have led to unjustified delays in treatment. This also might have introduced a bias with respect to symptom severity in our cohort, which was clearly skewed towards minor strokes as is reflected in the NIHSS score.

Secondly, the patient sample was very heterogeneous regarding stroke location with infra- as well supratentorial strokes as well as pronounced differences in lesion volumes. The latter might also be contributing to the missing evidence of detectable BBB leakage in the hyperacute stage.

Thirdly, the impact of various risk factors on BBB leakage must be interpreted with caution due to the small number of patients. This is despite the fact that the distribution of the number of risk factors was comparable across the different time groups.

A different and novel promising technique is currently emerging for assessing BBB leakage non-invasively: DP-pCASL (diffusion prepared pseudo-continuous arterial spin labeling) using the water exchange rate across the BBB as a means to describe BBB leakage. Shao et al.[21] found a good agreement analyzing BBB leakage using DCE-MRI and DP-pCASL as well as high test-retest reproducibility measuring water exchange rate immediately after and about 6 weeks later in elderly participants with cerebral small vessel disease. Using this technique with an endogeneous contrast agent that is water, more insight into the time course of BBB permeability under various conditions may be obtained.

Conclusion

Our results support the view of a continuous BBB leakage over the first 24 hours after stroke onset, in line with previously published experimental and human data. We found no association between cerebrovascular risk factors and BBB leakage.

Declarations

Authors contribution

K.V. conceived, designed the project, interpreted the data and wrote the manuscript. U.M., R.M, A.A.K. performed experimental and statistical analyses. A.A.K., J.B.F., CH.N. contributed to the content of this manuscript and interpretation of the data. All authors reviewed the manuscript.

Conflicts of interest

Jochen B. Fiebach: No relevant conflicts of interest. Outside the submitted work: JBF reports personal fees from Abbvie, AC Immune, Artemida, Bioclinica, Biogen, BMS, Brainomix, Cerevast, Daiichi-Sankyo,

Eisai, F.Hoffmann-La Roche AG, Eli Lilly, Guerbet, Ionis Pharmaceuticals, IQVIA, Janssen, Julius clinical, jung diagnostics, Lysogene, Merck, Nicolab, Premier Research, and Tau Rx.

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The other authors declare no potential conflict of interest.

Data availability

Data can be made available upon reasonable request.

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Figures

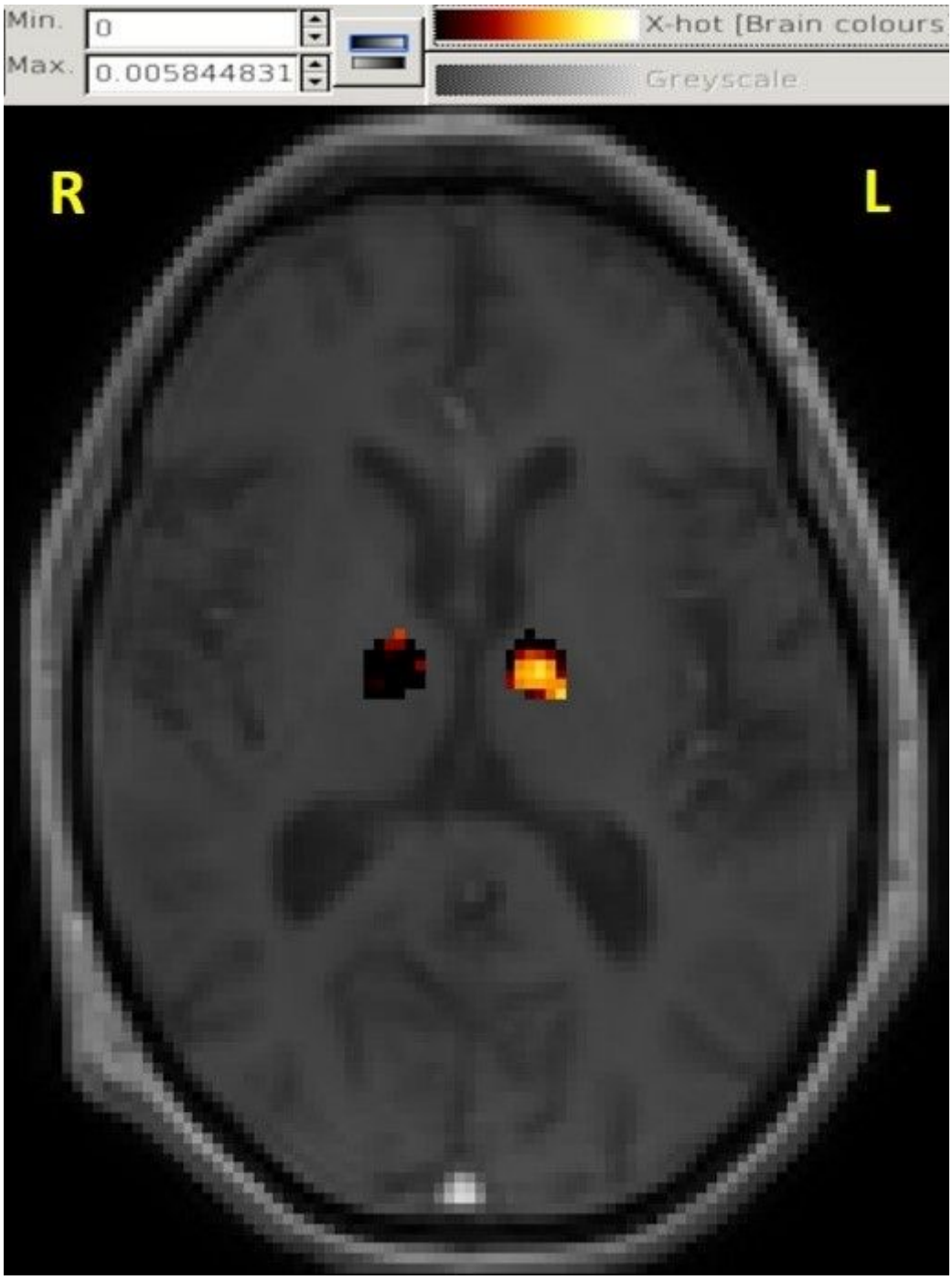

Figure 1

BBB leakage in an acute thalamic infarction Ktrans values in a stroke / mirror ROI of a left sided infarction of the thalamus.