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Multimodal MRI of experimental stroke

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Abstract

Stroke is the fourth leading cause of death and the leading cause of long-term disability in the United States. Brain imaging data from experimental stroke models and stroke patients have shown that there is often a gradual progression of potentially reversible ischemic injury toward infarction. Reestablishing tissue perfusion and/or treating with neuroprotective drugs in a timely fashion are expected to salvage some ischemic tissues. Diffusion-weighted imaging based on magnetic resonance imaging (MRI) in which contrast is based on water motion can detect ischemic injury within minutes after onsets, whereas computed tomography and other imaging modalities fail to detect stroke injury for at least a few hours. Along with quantitative perfusion imaging, the perfusion-diffusion mismatch which approximates the ischemic penumbra could be imaged non-invasively. This review describes recent progresses in the development and application of multimodal MRI and image analysis techniques to study ischemic tissue at risk in experimental stroke in rats.

Keywords

MRI; perfusion-diffusion mismatch; ADC; CBF; DWI; PWI; experimental stroke model; rodents; oxygen challenge; predictive mode; magnetic resonance imaging; rats; hyperperfusion; fMRI

Introduction

Stroke – the fourth leading cause of death and the leading cause of long-term disability [1] – is a medical emergency caused by a disturbance in the blood supply to the brain, resulting in loss of brain functions. There are about 800,000 new or recurrent stroke each year. More than 6 million Americans have permanent neurological deficits from stroke, and 71% of these stroke survivors can't return to work. Over $70 billion is projected to be expended on stroke patient care in 2011 [1]. The cost of stroke care is steadily rising because the conditions that put people at risk for stroke (such as heart disease, diabetes, and obesity) are also steadily on the rise. Despite the tremendous effort invested in stroke research, our ability to identify salvageable tissue and to minimize neurological deficit in stroke patients remains extremely limited. Recannalization by recombinant tissue plasminogen activator

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(rtPA) therapy is the only proven method that could salvage some brain tissue. rtPA treatment unfortunately is limited to only a small subset of patients because it has serious risk of often fatal hemorrhagic transformation and can only be administered within 4.5 hours of stroke onset [2]. As such, the ability to reliably distinguish salvageable versus nonsalvageable tissue remains a high priority for clinical decision making in the treatment of acute stroke.

In humans, the “perfusion-diffusion mismatch” obtained using magnetic resonance imaging (MRI) is presumed to approximate the “ischemic penumbra” and is increasingly used in clinical decision making in the management of acute stroke. Although the strict definition of ischemic penumbra requires correlation with energy metabolism [3–5] and such a correlation is not feasible in humans, the “ischemic penumbra” and viability thresholds have been operationally defined based on diffusion-weighted imaging (DWI), perfusion-weighted imaging (PWI) and equivalent modalities. Although “perfusion-diffusion” mismatch is widely observed in acute human stroke [6–10], the tissue fate characterized by the perfusion-diffusion mismatch remains poorly understood and controversial [11]. Consequently, clinical decision making based on perfusion and diffusion imaging has not yet reached its fullest potential. Animal models in which the perfusion-diffusion mismatch can be reproducibly studied under controlled conditions are important to fully characterize the tissue fate of ischemic injury (salvageable *versus* non-salvageable tissues) and to evaluate the efficacy of therapeutic intervention.

This paper reviews recent progresses, mostly from our group, in the development and application of multimodal MRI techniques and image analysis techniques to study ischemic tissue at risk in experimental stroke in rats. First, we review the use of perfusion or diffusion data to characterize acute ischemic stroke, and the use of automated clustering of combined perfusion and diffusion data to improve ischemic tissue characterization. Second, the compromise between high temporal and high spatial resolution for acute stroke imaging is described. Third, the blood-oxygen-level dependent (BOLD) fMRI of evoked responses to probe the perfusion-diffusion mismatch is described. Fourth, we describe quantitative predictive models to predict ischemic tissue fate based on acute perfusion and/or diffusion data. Fifth, we describe a multimodal MRI approach to investigate the hyperperfusion phenomenon associated with ischemic stroke. Finally, we review the use of BOLD fMRI of oxygen challenge to probe tissue fate and compare it to the perfusion diffusion mismatch.

Perfusion and diffusion MRI

MRI provides flexible and clinically relevant information to image stroke. In particular, DWI [12] in which contrast is based on water apparent diffusion coefficient (ADC) is widely recognized as a useful imaging modality, because of its ability to detect stroke within minutes after onsets, whereas computed tomography and other imaging modalities fail to detect stroke injury for at least a few hours. Hyperintense regions on DWI correspond to tissues with a reduced ADC of water. Although the biophysical mechanism(s) underlying ADC reduction remains poorly understood and controversial [12,13], the ADC decline has been correlated with energy failure and breakdown of membrane potential in animal models [3–5].

Cerebral blood flow (CBF) can be measured by using an exogenous intravascular contrast agent or by magnetically labeling the endogenous water in blood [14,15]. The most widely used perfusion MRI technique is based on dynamic susceptibility contrast imaging (see review [15]) in which an intravenous bolus of a blood-pool MR contrast reagent such as gadolinium is injected while T_2* or T_2 imaging is performed. This technique is generally performed only once due to recirculation of the contrast reagent and potential negative side effects. An alternative technique is based on the arterial spin labeling (ASL) technique that involves non-invasive magnetic labeling of blood water protons as they flow into the imaging slices, without the need for exogenous contrast reagents [15]. ASL is becoming increasingly popular for measuring CBF. The magnetically labeled water has a short half-life (~ blood T_1) and thus repeated ASL measurements can be made for signal averaging at relatively high spatial and temporal resolution. Continuous arterial spin labeling technique with the two-coil setup offers generally higher sensitivity than over single coil approach. A potential issue with ASL CBF is sensitive delayed transit time, which could underestimate CBF in and around the occluded territory. A few transit-time insensitive ASL techniques are under development [16].

ASL has been applied to evaluate the spatiotemporal progression of stroke rats during the acute phase [17,18]. The ADC and CBF maps delineate regions of hypointense abnormality. Areas with ADC reduction grow from 30 to 180 mins after ischemia, eventually reaching the CBF-defined lesion volume. In the permanent occlusion, ADC-defined lesion volume grows until it reaches CBF-defined lesion volume at about 180 mins [17,18], which correlated with the TTC infarct volume determined at 24 hrs. The ADC and CBF viability threshold in this rat stroke model is $0.53 \pm 0.02 \times 10^{-3}$ mm^2/s (30% ± 2% reduction) and 0.30 ± 0.09 mL/gram/mins (57% ± 11% reduction), respectively [17,18]. Reperfusion performed at 60 mins post occlusion demonstrates the “perfusion-diffusion” mismatch was salvaged, with ADC lesion volume at 180 mins reaching ~50% of the permanent occlusion group [18,19]. The degree of salvaged mismatch tissue is dependent on occlusion durations as expected [20]. Similarly, a few treatment drugs have also demonstrated to be effective in reducing infarct volume by salvaging the perfusion-diffusion mismatch defined by the viability thresholds in rat stroke models [21,22]. Quantitative diffusion and perfusion multi-slice imaging of the entire rat brain can now be acquired within a few minutes (~5 mins) and can be longitudinally performed to evaluate ischemic evolution.

Clustering approaches for delineating tissue fates

Analysis of the CBF-ADC scatterplot offers additional insight that is not readily evident by inspecting the ADC and CBF per se [17]. In the normal hemisphere, there is a single cluster with high ADC and CBF. In the ischemic hemisphere at 30 min, there are three clusters, namely: *i)* the “normal” cluster with normal CBF and ADC; *ii)* the “core” cluster with markedly reduced CBF and ADC; and *iii)* the “mismatch” cluster with reduced CBF but slightly reduced ADC. At 180 mins, essentially all the mismatch pixels migrated to the core in the permanent occlusion model. Upon reperfusion [19], the majority of the mismatch pixels and some core pixels returned to the normal. Tissue volumes, ADC and CBF values of each tissue cluster on the CBF-ADC scatterplots can be objectively determined using cluster analysis and each cluster can be mapped back onto the image spaces.

Automated cluster analysis of the CBF-ADC data has been used to objectively cluster pixels of different tissue fate. Shen et al. developed and applied an improved algorithm based on the automated ISODATA (self-organizing data analysis algorithm) technique [23] to characterize the spatiotemporal dynamic evolution of ischemic brain injury based on high-resolution, quantitative perfusion and diffusion measurements. In contrast to the normal left hemisphere, multiple clusters were resolved in the ischemic right hemisphere, corresponding to the “normal”, “at risk” (“perfusion-diffusion” mismatch), and “ischemic core” tissues. The unique advantage of the ISODATA is that the number clusters in the data set can be statistically determined, where other methods such as K-mean requires a priori assignment of the number of clusters. Tissue volumes, ADC, and CBF of each ISODATA cluster were quantified. Pixels of different ISODATA clusters were color-coded and mapped onto the image and ADC-CBF spaces. In some animals, essentially all the “perfusion-diffusion” mismatch pixels disappeared, while in other animals some mismatch pixels persisted at 180 minutes after occlusion. CBF of the “persistent mismatch” at 180 minutes was statistically higher than the CBF from the analogous region where the mismatch disappeared at 180 minutes. The ADC of the “persistent mismatch” did not decrease as ischemia progressed. In marked contrast, the ADC of analogous brain regions where the mismatch disappeared at 180 minutes decreased precipitously as ischemia progressed. Upon reperfusion, the majority of the mismatch pixels and some core pixels migrated to the normal clusters. Automated cluster analysis allowed objective classification of different tissue types and these tissue types can be mapped back onto the image spaces, providing a powerful and objective means for pixel-by-pixel visualization of different tissue fate. MRI is non-invasive and thus is ideally suited for longitudinal imaging in the same animals [24,25].

Spatial resolution versus temporal resolution

It is important to have fast imaging techniques with high spatial resolution to distinguish different tissue types in ischemic stroke. Partial volume effect (PVE) could hamper proper delineation of normal, ischemic, and at-risk tissues by blurring the boundaries among different tissue types and tissue viability. Visual delineation of ischemic lesions by manually drawing regions of interest (ROI) on the diffusion- and perfusion-weighted images is a common clinical practice and the presence of PVE could lead to significant errors in identifying ischemic tissue fates. In addition, it is conceivable that a substantial number of pixels with mild ADC and CBF reduction could arise simply from the physical effect of partial voluming, thereby confounding the interpretation of the operationally defined ischemic penumbra. High-resolution imaging could minimize tissue classification errors. Other advantages of high-resolution imaging include finer delineation of anatomic structures and increase in pixel density, which increases the statistical power of pixel-by-pixel cluster analysis, and reducing signal loss due to intravoxel dephasing. The drawbacks of higher spatial resolution are longer acquisition time and/or reduced SNR, which could also hamper efficacy of the imaging method. With improvement in parallel imaging and RF coils, faster and higher spatial resolution MRI protocols are expected.

Ren et al. evaluated ADC and CBF standard deviations in the normal left hemisphere were comparable between high and low resolution [26,27], despite increased noise and tissue heterogeneity at high resolution, substantial PVE was observed along the normal–abnormal

boundaries on the ADC and CBF maps, PVE resulted in overestimation of the abnormal tissue volumes at the expense of at risk and/or normal tissues, and misclassified pixels were quantitatively evaluated on a pixel-by-pixel basis, PVE appeared to be more severe at the early time points postischemia, and further reduction in spatial resolution and zero-filling resulted in more severe PVE. This study showed that there are some advantages to acquire stroke data at higher spatial resolution for the same scan time. Future study needs to evaluate whether the improved spatial resolution improves separation of different tissue types.

BOLD fMRI of perfusion-diffusion mismatch

In addition to anatomical MRI techniques based on tissue perfusion and diffusion, functional MRI of stroke animals can also be performed to evaluate the functional status of the "perfusion-diffusion mismatch." fMRI is a non-invasive imaging modality and has been widely exploited for mapping brain processes, ranging from perceptions to cognitive functions [28]. The most widely used fMRI technique is based on the BOLD signal or CBF signal. The BOLD contrast originates from the intravoxel magnetic field inhomogeneity induced by paramagnetic deoxyhemoglobin in red blood cells. Changes in regional deoxyhemoglobin content can be visualized in susceptibility-sensitized (i.e., T_2*-weighted) BOLD images. The BOLD fMRI technique is based on a principle discovered over 100 years ago [29] that neuronal activity is intricately coupled to cerebral blood flow. When a task is performed, regional blood flow increases disproportionally (which can be measured using the ASL technique), overcompensating the stimulus-evoked increase in oxygen consumption needed to fuel the elevated neural activity and, thus, resulting in a regional reduction in deoxyhemoglobin concentration. Thus, the BOLD signal increases following elevated activity relative to basal conditions, making it possible to dynamically and non-invasively map changes in neural activities.

fMRI applications to neurological diseases in animal models are emerging. We and others have previously demonstrated that bilateral forepaw somatosensory stimulation activated the somatosensory cortices of both hemispheres in a normal rat using isoflurane as the anesthetics [30,31] instead of the more common a-chloralose [32,33]. Recent development [30] also allows the addition of oxygen-consumption imaging to map oxidative metabolism and neural-vascular coupling in stroke rats. In the stroke rat 30 mins after occlusion, we demonstrated that activations in the somatosensory cortices were not detected in the ischemic hemisphere [20]. Functional MRI in stroke should be useful in determining whether risky therapeutic intervention should be performed if the "perfusion-diffusion" mismatch is already nonfunctional. Perfusion, diffusion and functional (including oxygen-consumption) imaging can be carried out within 30 mins at reasonably high spatial resolution.

Quantitative prediction of ischemic tissue fate

The ultimate goal of acute stroke imaging is to predict tissue fate based on acute MRI data. Sophisticated algorithms have been developed to predict ischemic tissue fate on a pixel-by-pixel basis. They included predictive models based on generalized linear model [34,35], probability-of-infarct [36,37], and artificial neural network (ANN) [38] and Support vector

machines (SVM) [39]. These predictive models provide statistical or probabilistic maps of infarct likelihood on a pixel-by-pixel basis utilizing only the acute MRI data. Performance analysis showed accurate prediction when compared with endpoint T2 MRI and/or histology. In addition, the effects of neighboring pixels and infarct incidence on prediction accuracy were also evaluated. Other potential a priori information can be incorporated in these predictive models. Prediction accuracy was quantified using receiver-operating characteristic (ROC) analysis.

Wu et al. predicted infarction in normal and hypertensive stroke rats subjected to embolic clot occlusion with and without rt-PA treatment at 1 hr after stroke using voxel-based generalized linear model algorithm [34]. They found that pre-treatment predicted outcome compared with post-treatment histology was highly accurate in saline-treated rats (92 ± 5%). Accuracy was significantly reduced in rt-PA treated animals (86 ± 8%). Animals that reperfused had significantly lower predicted infarction risk than nonreperfused animals, suggesting that tissue was more amenable to therapy. Shen et al. [36,37] documented the probability-of-infarct profiles of stroke rats underwent different MCAO durations. Using only acute ADC and CBF data, pixel-by-pixel prediction was made and compared to endpoint T2 imaging and histology. The AUCs were 87±3%, 90±4%, and 93±3% using ADC+CBF for the 30-min, 60-min and permanent MCAO, respectively. Huang et al. [38] used ANN prediction algorithms and found that the AUCs were 86 ± 3%, 89 ± 2%, and 93 ± 1% using ADC+CBF for the 30-min, 60-min and permanent MCAO, respectively. Adding neighboring pixel information and spatial information improved performance measures over ADC and CBF alone for the 60-min and 30-min MCAO group (88 ± 3% and 94 ± 1%, respectively) but only slightly for the permanent MCAO group (94 ± 2%). These differences were expected because permanent MCAO was less variable and ADC and CBF alone sufficiently accounted for prediction accuracy. ANN method performed slightly better than the probability of infarct method [37] operated on the same data sets although there were some minor methodological differences in how training groups were assigned.

Huang et al. used SVM prediction algorithms for predicting tissue fate and found that the AUCs were 86 ± 2.7%, 89 ± 1.4%, and 93 ± 0.8% using ADC+CBF for the 30-min, 60-min and permanent MCAO, respectively [39]. They found that CBF+ADC improved prediction accuracy. This is likely because CBF and ADC individually provided unique and relevant information. For example, in the presence of the perfusion and diffusion mismatch which would likely infract at later time points, neither ADC nor CBF data alone can capture such information. As such ADC would underestimate infarct volume while CBF overestimate infarct volume in this case. Moreover, perfusion deficit could overestimate final infarct volume if benign oligemia exists or reperfusion salvaged some tissue with initial perfusion deficit. Moreover, adding neighboring pixel information and spatial information markedly improved performance measures over ADC and CBF alone for the 60-min and 30-min MCAO group (94 ± 0.8% and 88 ± 2.8%, respectively) but again only slightly for permanent MCAO group (97 ± 0.9%). The improvement in SVM results was apparent when compared with ANN operated on the same data sets. Differences in animal stroke models (embolic *vs* suture), anesthetics (halothane *vs* isoflurane), and inclusion of slightly different types of MRI data (dynamic susceptibility contrast *vs* arterial spin labeling CBF) preclude

quantitative comparison with results reported by other research groups. Nonetheless, these quantitative prediction models (general linear model [34], probability-of-infarct method [37], ANN model [38] and SVM [39]) based on acute MRI data were overall accurate and yielded comparable AUC's on animal stroke models.

Hyperperfusion

Postischemic hyperperfusion (HP) – also known as 'luxury perfusion' or 'hyperemia' in which blood flow exceeds metabolic needs in the brain – has long been documented [40] to be a frequent, yet poorly understood, phenomenon. HP has been studied using positron emission tomography (PET) and magnetic resonance imaging (MRI) techniques in animal stroke models [41,42] and stroke patients [43,44]. Early postischemic HP sometimes observed immediately after recanalization is a hallmark of efficient recanalization after stroke [45,46] and it has been reported to be both beneficial (i.e., salvage tissue in and around the ischemic zone or prevent infarct growth) [47] and harmful (i.e., aggravate edema and hemorrhage, and neuronal damage from reperfusion injury) [48,49]. By contrast, late postischemic HP (48 hours after onset) is often associated with tissue necrosis [50–53]. Many studies have investigated the mechanisms underlying HP. Accumulated by-products (such as free radicals) could result in delayed neuronal death as well as production of vasoactive metabolites (such as lactic acid and adenosine) that could induce vasodilation through relaxation of vascular smooth muscle [54,55]. Some of these metabolites are implicated in modulating blood-brain barrier (BBB) permeability [56], potentially enhancing cerebral edema. Others have suggested neurogenic vasodilation [57] and passive physiologic coupling [47]. Histopathologcial investigation of stroke cat showed that late hyperperfusion in the necrotic core could in part reflects neovascularization with increased capillary density and endothelial hypertrophy [58]. However, the underlying spatiotemporal characteristics of postischemic HP and its progression with respect to other imaging markers (such as T1, T2, diffusion and contrast-enhanced MRI) remain incompletely understood. Most published non-invasive longitudinal studies to characterize HP had limited time points and terminal studies at different time points were confounded by inter-subject variations. Improved understanding of the HP spatiotemporal characteristics with respect to other imaging markers could improve understanding of stroke pathophysiology, which could ultimately lead to improved clinical stroke management.

Tanaka et al. examined how changes in tissue spin-lattice relaxation-time constant, blood brain barrier (BBB) permeability and arterial transit time affect CBF quantification by ASL and dynamic susceptibility contrast (DSC) in postischemic hyperperfusion in same rats {Tanaka, 2011 #3608}. Embolic stroke rats imaged 48hrs after reperfusion showed reliable regional hyperperfusion. ASL- and DSC-CBF of normal pixels linearly correlated whereas in ASL-CBF of hyperperfusion pixels were higher than DSC-CBF. T1 of hyperperfusion pixels were higher, transit time was shortened, and ΔR_2* time courses showed gadolinium diethylene*t*riamine*p*ent*a*cetate (Gd-DTPA) leakages in hyperperfusion regions. Hypercapnic inhalation, which does not change BBB permeability, showed overall CBF increase but ASL- and DSC-CBF remain linearly correlated. Mannitol injection, which increases BBB permeability, showed ASL-CBF to be higher than DSC-CBF. Tanaka et al. concluded that: *i)* under normal conditions the commonly used ASL and DSC provide comparable

quantitative CBF values, and *ii)* in ischemic hyperperfusion, T1 and BBB disruption were responsible for discrepancy in CBF measured by ASL and DSC.

Shen et al. also longitudinally evaluated the spatiotemporal dynamics of late HP in same animals subjected to 30-min, 60-min and 90-min intraluminal middle-cerebral artery occlusion (MCAO) in rats [59]. Multi-parametric MRI data including diffusion, perfusion, T_2, T_1, dynamic susceptibility-contrast MRI and MR angiography were acquired longitudinally at multiple time points up to 7 days after stroke. The spatiotemporal progression of HP was compared with T1, T2, diffusion, angiographic and BBB changes. The main findings were as followed. The early HP within 3H of recanalization was not detected in all three MCAO groups. The late (≥ 12H) HP was present consistently in the 30-min MCAO group, present in half of the animals in the 60-min MCAO group, and absent in the 90-min MCAO group. DSC CBF MRI and MRA independently corroborated HP detected by cASL. HP preceded T2 increase in some animals, and HP and T2 changes coincided in others. T2 peaked first at 24H whereas HP peaked at 48H post-occlusion, and HP resolved by day 7 in most animals at which point the arteries also became tortuous. HP was exclusively associated with poor outcome whereas tissue that was not infracted did not show HP.

Oxygen-challenge MRI

A novel approach was recently introduced to further probe tissue at risk by using T_2*-weighted MRI of transient oxygen challenge in ischemic stroke [60,61]. T_2*-weighted signal intensity is sensitive to relative concentration of deoxyhemoglobin [62,63]. The infarct core showed little or no change in T_2*-weighted signal intensity during oxygen challenge (OC). The at-risk regions surrounding the infarct core showed an exaggerated increase in OC T_2*-weighted signal intensity compared to the homologous region in the contralateral hemisphere. OC brings in oxygenated blood displacing the high deoxyhemoglobin concentration in the at-risk region where CBF is partially compromised but its metabolic activity remains significant. It was thus hypothesized that tissue with exaggerated increase in T_2*-weighted signal intensity during OC is potentially salvageable [60,61].

Shen et al. characterized of the effects of transient oxygen challenge on T_2*-weighted signal intensities in permanent stroke rats [64]. The major findings were as followed. The ischemic core cluster, derived automatically from perfusion and diffusion data, showed no significant response, whereas the mismatch cluster showed markedly higher percent changes relative to normal tissue in the acute phase. The exaggerated OC responses are more apparent in the primary somatosensory cortex than other brain structures at risk in this stroke model. Many of the mismatch pixels showed some exaggerated OC responses which became hyperintense on T_2-weighted MRI at 24 hrs. Basal T_2*-weighted signal intensities on the perfusion-diffusion contourplot were high in the normal cluster and low in the core cluster, with a sharp transition in the mismatch cluster. OC-induced changes on the perfusion-diffusion contourplot dropped as perfusion and diffusion values fell below their respective viability thresholds. *v*) Basal T_1 increased slightly in the ischemic core. OC decreased T_1 significantly in the normal hemisphere, indicative of hyperoxia-induced vasoconstriction or increased dissolved oxygen in the plasma, but OC had no significant T_1 effect in the

ischemic core and mismatch pixels. *vi*) OC decreased CBF significantly in the normal hemisphere, but had no significant CBF effect on the mismatch and the ischemic core pixels.

CONCLUSIONS AND PERSPECTIVES

This review summarizes our recent works on multimodal MRI of the perfusion-diffusion mismatch. The combined use of perfusion, diffusion, physiological and functional MRI and image analysis methodologies provides powerful tools to improve characterization of cerebral ischemia, to longitudinally monitor ischemic progression, evaluation of drug efficacy, and statistically predict ischemic tissue fate. Animal stroke models in which the "perfusion-diffusion mismatch" and its functional status can be reproducibly studied under controlled conditions are highly valuable for establishing exploring novel MRI modalities and to characterize ischemic tissue fate. A major confound of animal stroke studies is the need for anesthetics which could have undesirable (i.e., neuroprotective) effects. However, with the use of proper controls and careful analysis, this confound can be mitigated or at least minimized. Translations of these methodologies require vigorous testing and improvement in temporal efficiency of these MRI protocols. Non-invasive MRI methodologies can be readily be applied to study stroke in high-order animals, such as non-human primates [65,66], as well as in humans.

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