Early metabolic biomarkers identifying permanent stroke in mouse brain using ^{1}H MRS

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INTRODUCTION

Recent mouse models in stroke have shown that localized ^{1}H MRS capable of studying evolution of numerous brain metabolites after transient cerebral ischemia (1) and consequently identifying early predictive markers for lesion (2,3). Glutamine and the sum of NAA, Tau and Glu could be applied to identify different degrees of transient ischemic damage as early as 3hr after insults (2,3). We thought longitudinal ^{1}H MRS studying permanent stroke would help understanding its cascading damages in brain tissue and thus could help classifying stroke types, from transient ischemic attack with no lesion (TIA), minor stroke, moderate stroke and severe stroke without reperfusion. Therefore, the aim of this study was to study the evolution of brain metabolites after permanent focal ischemia in mice using ^{1}H MRS at 14.1T and identifying possible biomarkers.

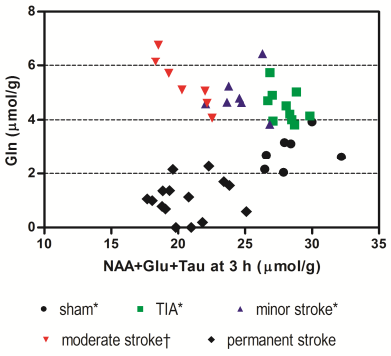

Figure 2. Scatter plot of glutamine over sum of NAA, Tau and Glu measured at 3hr after operation. '*' indicates data were from previous studies, (2,3) and '†' from Lei et al. 2009 (1).

METHODS

All animal studies were approved by the local veterinary authority. Adult male ICR-CD1 mice (n=25) underwent focal ischemia as previously described except removing inserted filaments. To minimize the effect of isoflurane on animals, MR studies were randomly performed at 30-45min, 3hr, 8hr and 24hr after permanent focal ischemia to reach 6-10 mice per time-point. At 14.1T, immediately after T_2-weighted (T2WI) images (TE_{eff}/TR=50/5000ms) were acquired and field homogeneities were improved (water linewidths <25Hz), localized ^{1}H MRS was applied on ipsilateral striatum (~5.6μL) using SPECIAL (TE/TR=2.8/4000ms, NT=240-320, SNRs>10, 1 and references therein). The water signals with no water suppression (NT=8) were acquired for further absolute quantification. MR spectra were processed and quantified using LCModel (1 and references therein). The water content was assumed 80% absolute quantification. Brain metabolites were found detectable with CRLB <50%. Sham operated, TIA and minor stroke were taken from previous studies (2,3) and moderate stroke was from lei et al. 2009 (1).

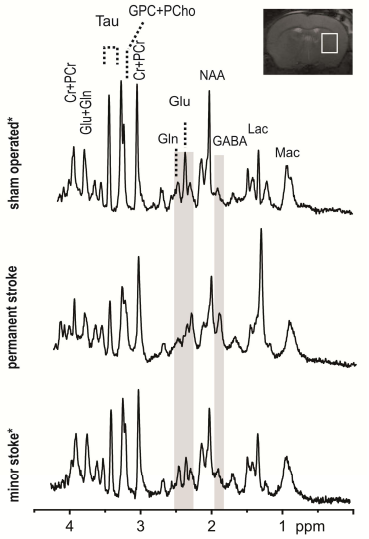

Figure 1 Typical localized ^{1}H MR spectra of mouse striatum (square in T2WI image) 3hr after sham, minor stroke and permanent stroke at 14.1T. '*' indicates data were from previous studies, (2,3). Noticeable differences in Glu, Gln and GABA between three groups were highlighted in grey shaded areas.

RESULTS AND DISCUSSION

T2WI images presented sufficient anatomical structures allowing localization of the ipsilateral striatum (Figure 1). The T2WI images showed slightly abnormal T_2-hyperintensities 3hr after permanent stroke while ADC maps in the ipsilateral striatum and cortex were consistently lower than those in the contralateral side (data not shown). Noticeably, MR spectra at 3hr presented highly increased GABA and decreased glutamine, which were different from those of transient ischemic attacks, i.e. elevated glutamine (Figure 1). When plotting glutamine contents against NAA+Glu+Tau (mostly locating in neurons) obtained at 3hr after operation, data from permanent stroke were clearly separated from four other groups, sham operated, TIA, minor and moderate stroke (Figure 2). In addition, we notice that GABA elevated immediately after permanent stroke, i.e. shortly after operation (Figure 3). This is consistent with previous studies (4 and references therein). Therefore, we could identify different stroke types based on metabolites measured by means of ^{1}H MRS. It is interesting to note that the degradations of selected metabolites, such as NAA and Tau, were close to a mono-exponential decay (R^2>0.9) except a bi-exponential evolution in GABA (Figure 3). This could be explained by different mechanisms for these metabolites evolving after permanent stroke. On top of that, the progressive decay could possibly help identifying stroke onset time, a major issue in a large fraction of patients with unknown stroke onset time. In conclusion, studying metabolites using ^{1}H MRS allowed differentiating various stroke subtypes and thus could improve diagnosis and treatment selection in clinics.

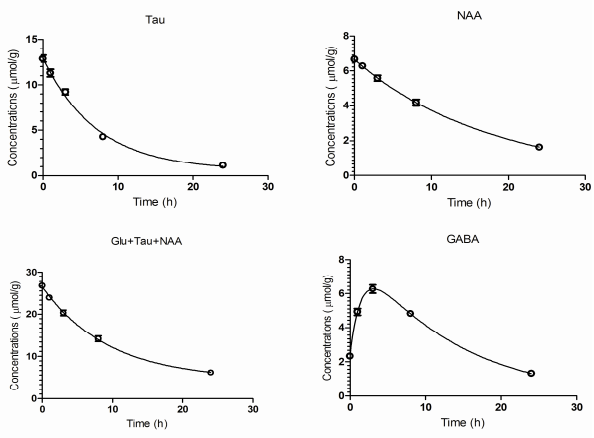

Figure 3 Evolution of selected metabolites after permanent occlusion. The curved lines were the fitting results for the degradations of Tau, NAA and Glu+Tau+NAA, i.e. mono-exponential decay. Alternatively GABA was fitted bi-exponentially. Error bars are SEMs.

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ACKNOWLEDGMENTS Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL,UNIGE, HUG, CHUV, EPFL, SNF 3100A0-122498, and the Leenaards, Jeantet and Gianni Biaggi de Blasys Foundations.