Localized ^{1}H MRS of mouse brain in vivo reveals metabolic markers predicting the development of ischemic damage

H. Lei[1,2], C. Berthet[3], L. Hirt[3], and R. Gruetter[1,4]

[1]EPFL, Lausanne, Vaud, Switzerland, [2]UNIL, Lausanne, Vaud, Switzerland, [3]CHUV, Lausanne, Vaud, Switzerland, [4]UNIGE, Geneva, Geneva, Switzerland

INTRODUCTION Studies in animal models help understanding the mechanism underlying ischemic changes in the brain parenchyma and could lead to identify biomarkers useful for diagnosis or treatment in stroke patients. Previous studies suggested that localized ^{1}H MRS allowed revealing neurochemical profile in mice at high magnetic field (1). Studies in ischemic mice suggested that quite a few metabolites, such as glutamine (Gln), glutamate (Glu), N-actylaspartate (NAA) and lactate (Lac), were altered as early as at 3 hours post insults (2). We sought to investigate which of these changes reflected irreversible tissue damage by comparing the effects of minor and more severe transient focal ischemia.

METHODS

All experiments were approved by the local veterinary authorities. ICR-CD1 mice (~25g) were subjected to 10-minutes (n=11) or 30-minutes (n=9) endoluminal middle cerebral artery occlusion (MCAO) by the filament technique at 0-hr (2). The regional CBF was monitored in all animals, by laser-Doppler flowmetry with a flexible probe fixed on the skull, with <20% of baseline CBF during ischemia and >70% during reperfusion. Thereafter, animals were maintained in an incubator at 31°C. All MR studies were carried on in a horizontal 14.1T magnet. A home-made quadrature coil was used as RF transceiver. The animals were anesthetized under 1~2% isoflurane for the studies while physiological parameters, such as temperature and breathing were monitored. Fast spin echo images with T_2 hyperintensive wighted (TE/TR=50/6000ms) were acquired to localize the volume of interest (VOI) and evaluate the lesion size. Immediately after adjustment of field inhomogeneities, localized ^{1}H MRS was applied to obtain the neurochemical profile in the striatum (6-8μL VOI) (1, 2, 3). Absolute quantification was done with 80% water as reference using LCModel (4). Six sham animals (group A) underwent identical procedures except for the MCAO. Two-way ANOVA (GraphPad Prizm) was applied for statistics analysis.

RESULTS AND DISCUSSION Shimming resulted in high quality spectra (Figure 1) at 3-hr after ischemic insults with 20±5 Hz linewidths and 15±3 in SNRs. Consequently, the obtained neurochemical profile resulted in the determination of 19 metabolites with CRLB<35% (Figure 2). The mild 10-min ischemia insults resulted in two sub-groups, no lesion (group B, n=7, Figure 1B) or lesion (group C, n=4, Figure 1C) respectively, as judged from the T_2 hyperintense images acquired one day post ischemia (Figure 1). The 30-min ischemic insults (group D) consistently induced a mainly striatal lesion (Figure 1D). In all ischemic groups (B, C and D), a common change was as an increase in Gln (Figure 1), which most likely reflected the ischemia-induced glutamate release into the extracellular followed by astrocytic uptake and metabolism (5). NAA and Glu were reduced in group C and D but not in group A or B (Figure 2) therefore correlating with lesion development shown on T_2 hyperintense images at 24-hr. When plotting Gln as a function of total NAA and Glu (Figure 3), NAA and Glu at 3-hr post insults are potential markers for lesion development. Taking into account that both NAA and Glu are mostly neuronal (6, 7), our data suggest that the neurochemical profile identifies early neuronal changes at 3-hr which reflect irreversible damage with T_2 signal changes seen at 24-hr. In conclusion, the neurochemical profile identifies early metabolic response indicating the degree of ischemic damage.

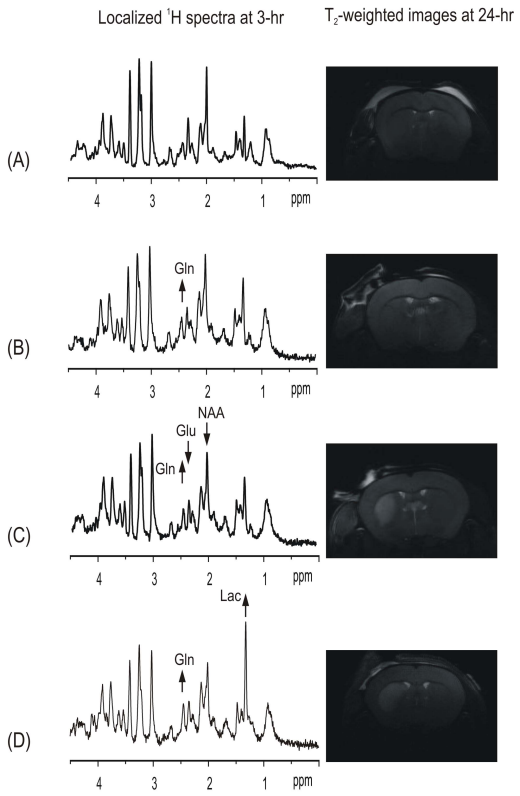

Figure 1 Typical spectra (left column) and T_2-weighted coronal images at bregma 0-mm (right column). Each panel represents data from an individual group, (A) sham; (B) 10-min MCAO no lesion; (C) 10-min MCAO with lesion; (D) 30-min MCAO. Some apparent changes are marked with the oriented arrows.

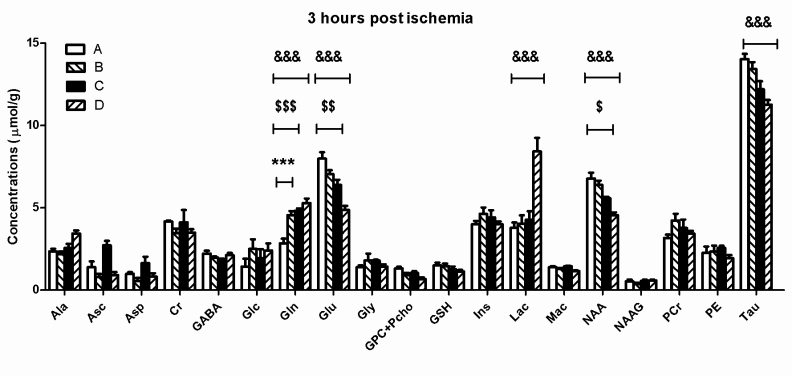

Figure 2. Comparison of the neurochemical profiles of the ischemia insult groups to that of the sham group with error bars as SEMs. Different symbols indicate the significant difference in group B ("*"), group C ("$") and group D ("&") groups (Figure 1) when comparing to group A. The increment of symbol numbers represents the increase of significant levels with p-value from 0.05, 0.01 to 0.001.

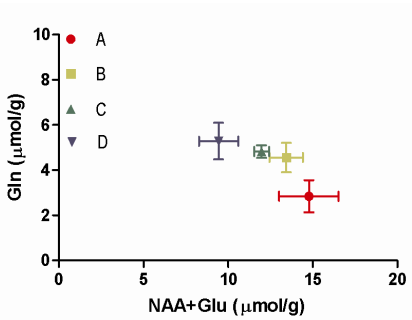

Figure 3. Direct visualization of Gln concentration in each group (A sham; B 10-min MCAO no lesion; C 10-min MCAO with lesion; D 30-min MCAO. As in Figure 1 and methods) as a function of NAA+Glu contents. Error bars are SDs.

References:

1. Tkac I et al. J Neurochem 2007 100 :1397
2. Lei H et al. Proc Intl Soc Mag Reson Med 16, 1941
3. Mlynarik V et al. Mag Reson Med 2006 56:965
4. Provencher S Mag Reson Med 1993 30:672
5. Benveniste H et al. J Neurochem 1984 43 :1369
6. Demougeot C et al. J Neurochem 2004 90 :776
7. Ottensen OP et al. Neurosci 1992 46 :519

Acknowledgements:
Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL,UNIGE, HUG, CHUV, EPFL and the Leenaards, Gianni Biaggi-de Blasys and Jeantet Foundations