Biomarkers to estimate the time of onset of cerebral ischemia

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PURPOSE The objective of this study was to determine whether ^{1}H MRS of the ischemic striatum could provide useful information on the estimation of the onset time of cerebral ischemia.

METHODS We modeled ischemic stroke in male ICR-CD1mice (n=40) using a permanent middle cerebral artery filament occlusion model (pMCAO) with laser Doppler control of the regional cerebral blood flow, <20% of the baselines during ischemia. Consequently, 36 mice (4 were excluded because of unsatisfactory blood flow drops, i.e. >20% of the baselines during ischemia) were subjected to repeated MRS measurements of ipsilateral striatum (6-8µl) using a home-built quadrature coil (two-13mm-diameter loops) in a 14.1T horizontal bore MRI. Immediately after T_2-weighted MR images and adjustments of field inhomogeneities, ^{1}H MRS was acquired from the target regions. The acquired MR spectra were processed and quantified referencing to striatal water contents, as previously[1, 2]. The data over the entire of studies were analyzed and the most applicable evolution models were estimated. In order to evaluate feasibility of estimation of ischemic onset time, 4 mice were prepared with satisfactory requirements (as above) in a blind manner to the person who acquired and interpreted the ^{1}H MR spectra.

RESULTS AND DISCUSSION We observed different spectral patterns after permanent ischemia than after transient ischemia, with an initial striking increase in γ-aminobutyric acid (GABA) and no increase in glutamine (Gln) (Fig. 1). We observed a mono-exponential decline in several metabolites such as taurine (Tau), N-acetyl–aspartate (NAA) and to some extent the sum of Tau, NAA and glutamate (Glu) (Fig 2 a, b, d). Using these decline curves we were able to estimate the time of onset of permanent ischemia in four mice the blinded experiment with an accuracy of approximately ±30 min (Table 1). It is also interested to note that the Tau reduction of one mouse (GRGR) was 2.3%, significantly lower than the measurement errors (~8.5%) from healthy mice, consequently the estimated onset time of this mouse using Tau only was beyond 1 hour (Table 1). However, the sharp increase of GABA within 2-hour after pMCAO (Fig 1 and 2c) and other metabolite changes might improve the estimated onset time of this mouse. For example, the estimated onset time using GABA was 10:55, which was the ischemia onset time of this mouse (Table 1). Alternatively, we also observed that acetate (Ace), one of degradation products from NAA, elevated after pMCAO (Figure 3e). The detection of acetate after pMCAO using ^{1}H MRS was confirmed with a moderate echo approach (data not shown here). Consequently, a linear increase of Ace/NAA (Fig 2f) was observed and could be possible to differentiate ischemic windows. Indeed, when we plot Tau against Ace/NAA, all 4 mice were within 4.5hr after ischemia (Fig. 3a).

CONCLUSION This is a novel approach, in mice, addressing the clinically highly relevant problem of determining the time of onset of ischemic stroke in stroke patients.

REFERENCES 1)Berthet C et al. *Stroke*. 2011;42:799-805 2) Lei H et al. *J Cereb Blood Flow Metab*. 2009;29:811-819

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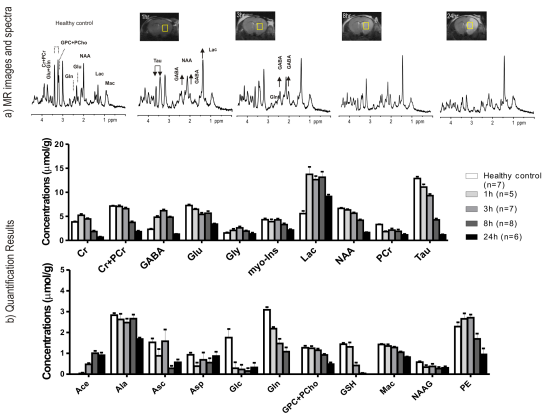

Figure 1 Representative T_2-weighted MR images and corresponding MR spectra (a) from permanent MCAO (pMCAO) and control mice at selected time points. Quantification of results is shown in (b). Healthy control[1,2](white bars), 1h (light gray bars), 3h (gray bars), 8h (dark gray bars) and 24h (black bars). Error bars are SEMs.

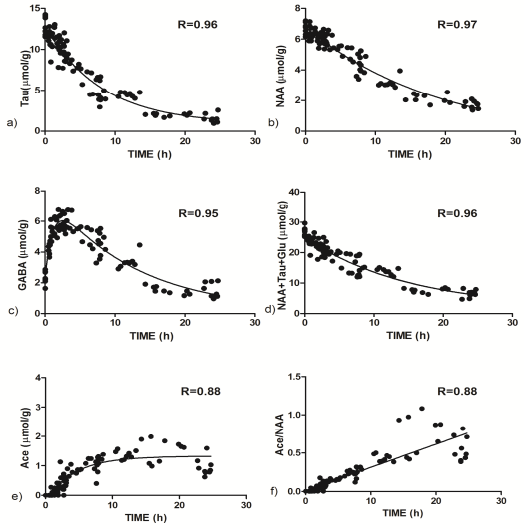

Figure 2 Selected metabolite evolution patterns after p MCAO (Tau, a); NAA, b); GABA, c); NAA+Tau+Glu, d); Ace, e) and Ace/NAA, f)). Black dots and solid lines represent all data and the corresponding best fit non-linear plots. The resulting R-values are reported.

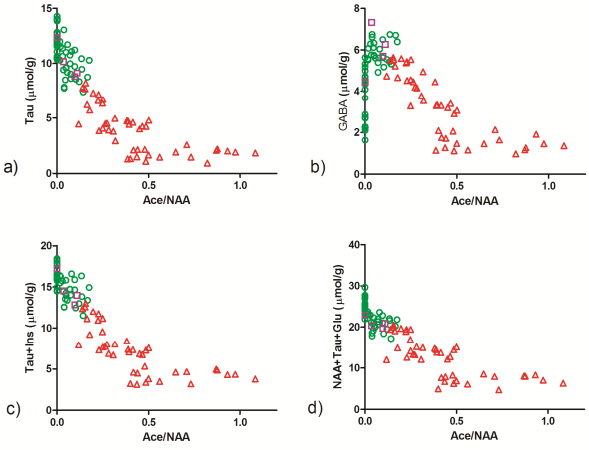

Figure 3 Scatter plots of selected metabolite concentrations (a), Tau; b), GABA; c), Tau+Ins (myo-inositol); d), NAA+Tau+Glu) against the Ace/NAA ratio after p MCAO and within the therapeutic time window, 0-4.5h (green open circles) or outside, >4.5h (red open triangles). The purple open squares represent 4 animals of unknown occlusion time to the graph plotter.

Animal ID	T_{Tau}	$T_{NAA+Tau+Glu}$	T_{NAA}	PO TIME	MR TIME	Estimated ΔT_{Tau}
GRGR	12:09± 00:10	10:54±00:19	8:04±01:41	10:55	12 :23	1:14
RBRB	10:33±00:23	10:27±00:24	8:15±01:57	10:10	13 :15	00:23
NRBN	11:08±00:26	10:31±00:29	10:36±01:36	11:31	14 :08	00:23
NGBN	13:28±00:27	15:24±00:26	12:31±00:47	13:01	15 :17	00:27

Table 1 Estimation of the ischemia onset times (PO TIME) using decay of NAA (T_{NAA}), Tau (T_{Tau}) and NAA+Tau+Glu ($\Delta T_{NAA+Tau+Glu}$) and incorporating errors of the estimated time difference in Tau (ΔT_{Tau}). PO permanent occlusion.