Biomarkers to estimate the time of onset of cerebral ischemia

Carole Berthet1, Lijing Xin1, Corine Benak1, Rolf Gruetter1,2,3, Lorenz Hurt1, and Hongxia Lei1,4

1Department of Clinical Neurosciences, Centre Hospitalier Universitaire Vaudois, Lausanne, Vaud, Switzerland, 2LIFMET, Ecole Polytechnique Fédérale de Lausanne, Vaud, Switzerland, 3University of Lausanne, Vaud, Switzerland, 4CRBM-AIT, Ecole Polytechnique Fédérale de Lausanne, Vaud, Switzerland, 5University of Geneva, Geneva, Switzerland

PURPOSE
We modeled ischemic stroke in male ICR-CD1 mice (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 (t-8ul) using a home-built quadrature coil (two-13mm-diameter loops) in a 14.1T horizontal bore MRI. Immediately after T2-weighted MR images and adjustments of field inhomogeneities, 1H MRS was acquired from the target regions. The acquired MR spectra were processed and quantified referencing to striatal water contents, as previously. The data over the entire 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 1H 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 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 interesting 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 1H 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 patients.

REFERENCES

ACKNOWLEDGEMENTS: This work was supported by the Centre d’Imagerie BioMedicale of the University of Lausanne (UNIL), University of Geneva (UNIGE), Hôpitaux Universitaires de Genève (HUG), Centre Hospitalier Universitaire Vaudois (CHUV), and Ecole Polytechnique Fédérale de Lausanne (EPFL); and the Leenaards, Jeantet and Gianni Biaggi de Basly’s Foundations.

Figure 1 Representative T2-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 (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 pMCAO (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 pMCAO 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.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>(\Delta T_{Tau})</th>
<th>(T_{NAA+Tau+Glu})</th>
<th>(T_{NAA})</th>
<th>(T_{NAA} ) (\Delta T_{Tau})</th>
<th>PO TIME</th>
<th>MR TIME</th>
<th>Estimated (\Delta T_{Tau})</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRGR</td>
<td>12.09±00:10</td>
<td>10.34±00:10</td>
<td>8.04±01:41</td>
<td>10.55</td>
<td>12.23</td>
<td>15.15</td>
<td>00:23</td>
</tr>
<tr>
<td>RBBB</td>
<td>10.32±00:23</td>
<td>10.27±00:24</td>
<td>8.15±01:37</td>
<td>10.18</td>
<td>15.15</td>
<td>00:23</td>
<td></td>
</tr>
<tr>
<td>NRBN</td>
<td>11.08±00:26</td>
<td>10.31±00:29</td>
<td>10.30±01:36</td>
<td>11.31</td>
<td>14:08</td>
<td>00:23</td>
<td></td>
</tr>
<tr>
<td>NGNB</td>
<td>13:28±00:27</td>
<td>15:24±00:26</td>
<td>12:31±00:47</td>
<td>13:01</td>
<td>15:17</td>
<td>00:27</td>
<td></td>
</tr>
</tbody>
</table>

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 \(\Delta T_{Tau}\), PO permanent occlusion.