Correlation of Proton Transfer Ratio and Intracellular pH in Acute Ischaemia

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Introduction

Acidification is a common consequence of anoxia and ischaemia. Low tissue pH has been shown to worsen tissue damage caused by transient ischaemia [1]. Thus, it would be beneficial to have imaging methods for intracellular pH (pH_i) to assess tissue status in cerebro-vascular conditions, including ischaemia. Change in proton transfer ratio (PTR) between the proton amide groups and water is shown to reflect ischaemia due to acidosis and thereby enable a way to image pH_i in brain [2]. In the present study we investigated acutely ischaemic brain by diffusion, $T_{1\rho}$ and PTR MRI and quantified brain lactate by MRS to estimate pH_i using the established correlation between cerebral lactate and pH_i [3].

Methods

Male Wistar rats (280 to 340g, n=12) were anesthetized with 1% halothane in 70/30 N_2O/O_2 for middle cerebral artery occlusion (MCAo) (n=8) [4] or sham operation (n=4) and MRI. Arterial blood gases and pH were analyzed during experiments and physiologic homeostasis was maintained as required. MRI experiments were performed in a horizontal 4.7T magnet interfaced to a Varian Inova console during 60 minutes of MCAo and reperfusion (MRI scans performed 60, 90, 120 and 150 min from the onset of MCAo). A quadrature half-volume coil was used as a transmitter and receiver. Diffusion, $T_{1\rho}$ and Z-spectroscopy data were acquired in an interleaved manner using a linescan spin-echo sequence (TE 15 ms, line 3mm*3mm*35mm, 128 pixels along the line). The imaging line was positioned so that the centre of the coronal line was 4 mm from the surface of the brain and 4 mm caudally from the olfactory bulb covering the striatum and parietal cortex. D_{av} was quantified using a spin echo sequence incorporating four bipolar gradients along each axis with four b-values from 0 to 1370 s/mm^2 (TR 2.5 s, TE 55 ms). The on-resonance $T_{1\rho}$ was quantified using five adiabatic spin-lock pulses ranging from 10 to 90 ms with a B_{1SL} = 0.6 G (TR 2.5 s, TE 15 ms). The Z spectra were acquired by saturating off-resonance frequencies with a train of 6.6 ms long 180˚ Gauss pulses with interpulse delay of 3.4 ms for 4 s (offset frequencies of 0-2 kHz, TR 7 s, TE 15 ms). Change in the PTR between ipsilateral and contralateral side (ΔPTR) was calculated as described by Zhou et al.[2] Water suppressed ^{1}H spectra was measured from four ischaemic and four control animals using LASER-sequence (TR 4 s, TE 30 ms) with a localized 3 x 3 x 4 mm^3 voxel positioned in the area of ischaemic striatum confirmed by diffusion MRI. Lactate concentration was calculated from lactate and NAA peaks, analyzed with jMRUI, using NAA concentration of 7.2 mmol/kg [5]. The lactate content (x) was then transformed to pH_i (y) by a linear fit of y=-0.0335x+6.83 [3].

Results

D_{av} declined by >25% in the ischaemic tissue by 60 minutes of MCAo (Fig 1, Table I). Cerebral $T_{1\rho}$ value had increased by 9 % by 60 minutes of MCAo, continuing to increase over the entire observation time (Fig 2, Table I). The ΔPTR was greatly decreased at 60 minutes from MCAo compared to controls, followed by a gradual return towards a value of the controls after reperfusion (Fig 3, Table I). pH_i derived from lactate content was 6.81±0.01 in the control brain, a value that is about 0.2 pH units less than rat brain pH_i commonly found by ^{31}P NMR spectroscopy. A plot of ΔPTR vs. pH_i showed a strong correlation (P<0.001) in the ischaemic brain (Fig 4). It is worth noting that the correlation between pH_i and $T_{1\rho}$ in the same conditions was less evident (p=0.04) (graph not shown).

Conclusions

$T_{1\rho}$, diffusion and PTR apparently provide complementary information from the brain exposed to MCAo that may facilitate characterization of tissue viability and damage progression. The present results indicate that PTR shows deep asymmetry in early moments of ischaemia and this asymmetry has a strong correlation with the brain lactate content. Thus, it is a reasonable working hypothesis, that one of the key pathophysiological factors affecting PTR during ischaemia is cellular acidification.

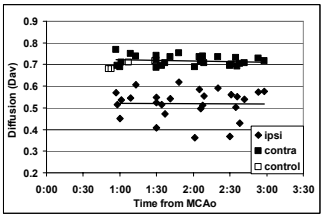

Fig.1: D_{av} values of ipsilateral and contralateral side

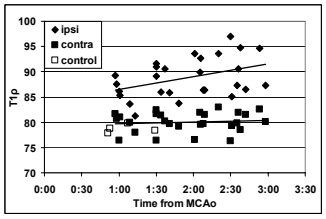

Fig.2: $T_{1\rho}$ values of ipsilateral and contralateral side

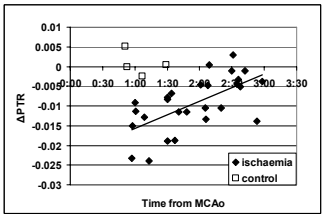

Fig.3: ΔPTR plotted vs. time

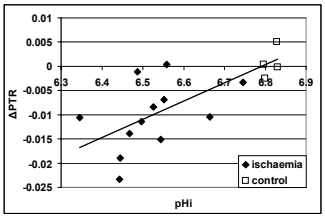

Fig.4: ΔPTR plotted vs. pH_i

Table I: Measured and calculated parameters from different time points (mean ± SEM). Significance between ipsilateral and contralateral values was tested by Student's t-test (*P<0.1, **P<0.01).

	Diffusion (D_{av}) [*10^{-3} mm^2/s]		$T_{1\rho}$ [ms]		ΔPTR	pH_i
	Ipsi	Contra	Ipsi	Contra	(Ipsi-Contra)	
Control	0.70 ± 0.01	0.69 ± 0.00	78.7 ± 0.4	78.9 ± 0.2	0.001 ± 0.002	6.81 ± 0.01
60min	0.54 ± 0.02**	0.73 ± 0.01	85.5 ± 1.2**	79.6 ± 0.8	-0.016 ± 0.003**	6.49 ± 0.03**
90min	0.52 ± 0.02**	0.72 ± 0.01	88.3 ± 1.2**	80.1 ± 0.8	-0.012 ± 0.002**	6.51 ± 0.03**
120min	0.52 ± 0.03**	0.72 ± 0.01	89.6 ± 1.4**	80.2 ± 0.8	-0.006 ± 0.002*	6.52 ± 0.09*
150min	0.51 ± 0.03**	0.71 ± 0.01	91.1 ± 1.6**	80.1 ± 0.8	-0.005 ± 0.002*	6.57 ± 0.16*

References

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Acknowledgements

Supported by the Sigrid Juselius Foundation and the Finnish Cultural Foundation of Northern Savo