The Response to Acute Hypoxia and Infection Studied by MRI/MRS in a Chicken Embryo Model of Perinatal Brain Injury

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Introduction Perinatal brain injury is commonly multifactorial in origin. We have previously reported that in the chick embryo in-ovo histological evidence of neuronal cell death is more severe following a combination of hypoxia and exposure to bacterial endotoxin (a model of infection) than for each factor alone[1]. The two main aims of this study were firstly, to use magnetic resonance to assess the acute disturbance of cerebral metabolism, water diffusion, blood volume and blood oxygenation in response to hypoxia, and secondly, to determine if the acute response was altered by pre-treatment with bacterial endotoxin.

Methods Nineteen white Leghorn chicken eggs (55-65g) were incubated at 38°C and 55-70 % humidity. On incubation day 19, chick embryos received 0.4 ml of either saline (n=10) or lipopolysaccharide (LPS, 3mg, n=9) from Salmonella Typhimurium 4 hours prior to transient hypoxia (FiO_2=0.04, 60 minutes) which was initiated whilst in the bore of a 7T Bruker Spectrometer. All the embryos received 0.5mg of tubocurarine dropped onto the chorioamniotic membrane for immobilization during data acquisition. The periovo environment was controlled to maintain gas flow, oxygenation and temperature (38°C). The following MRI/MRS measurements were repeated approximately every 15 minutes before, during and post hypoxia: proton spectroscopy, quantitative diffusion weighted imaging (an indicator of metabolic energy status), and quantitative T_2 and T_2* imaging (reflects changes in blood oxygenation and volume). MR parameters are displayed in Table1. Metabolite peak-area ratios were calculated from the spectra using LCModel[2]. Apparent water diffusion coefficient (ADC) maps were calculated for each diffusion direction (x/y/z) and from these 1/3 Trace images (Dav) were obtained (see Figure 1). Additionally, both T_2 and T_2* maps were calculated. For ADC, T_2 and T_2* maps a region was drawn covering the whole brain for each image and the mean value and approximate time of scan recorded. These values were then grouped according to time interval relative to the start of hypoxia. The average responses of each group (hypoxia only and hypoxia + LPS) were obtained for each time interval. Additionally, the measured values were tested for intergroup (hypoxia / hypoxia + LPS) and interperiod (baseline / hypoxia / post-hypoxia) differences using a multifactor analysis of variance (ANOVA).

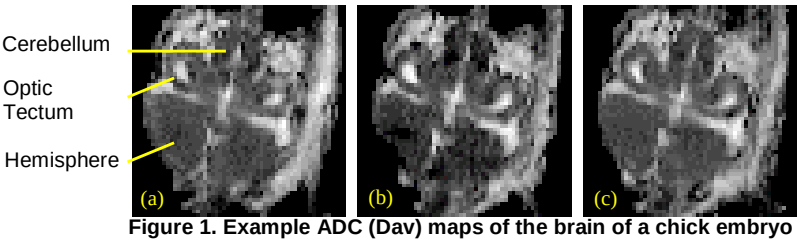

Figure 1. Example ADC (Dav) maps of the brain of a chick embryo (a) before, (b) during and (c) post hypoxia

	Method	Parameters
T_2*	FLASH	TR=100ms, TEs=6.3 (1nex), 23.4 (3nex), 33.4ms (3nex), FoV=$(35mm)^2$, 128x128 matrix
T_2	EPI	TR=2500ms, TEs=73 (5nex), 110ms (10nex), FoV=$(35mm)^2$, 128x128 matrix
ADC	EPI	TR=2500ms, TE =119ms, FoV=$(35mm)^2$, 128x128 matrix, b=0 (12nex), b=875_x(30nex), b=875_y(30nex), b=875_z(30nex)
^{1}H spectra	PRESS	TR=2000ms, TE =135ms, $(4mm)^3$ voxel positioned at brain centre, 128nex

Table 1. MR parameters

Results Compared to baseline values lactate (Lac) / total creatine (Cr) had increased significantly by the end of hypoxia (60 mins) (see Table 2) and then returned close to baseline by 120-minute post hypoxia. Similar findings were observed in both groups. N-acetylaspartate (Naa) peak-area ratios did not change significantly with respect to hypoxia or experimental group (values not given). Global ADC fell significantly (typically by 10-15% baseline values) during hypoxia (see Figure1) and then returned to baseline values within 30 minutes of the end of hypoxia. There were no significant intergroup differences between baseline ADC or size of ADC reduction during hypoxia. T_2 and T_2* also fell during hypoxia and then returned to baseline. There was no significant T_2 difference between the experimental groups. However, compared to the hypoxia only group T_2* was significantly reduced ($p<0.05$) in the LPS + hypoxia group before, during and after hypoxia. In addition, the T_2* decrease during hypoxia was significantly attenuated ($p<0.05$) in the LPS + hypoxia group. These results are summarised in Table 2.

HYPOXIA ONLY	baseline	hypoxia end	2 hours post hypoxia
T2 (ms)	70.2 ± 5.1	58.0 ± 3.6*	70.7 ± 3.9
T2* (ms)	48.9 ± 7.9†	31.7 ± 10.3*†	44.6 ± 6.7†
Dav ($1x10^{-3}$ mm^2/s)	0.96 ± 0.08	0.84 ± 0.07*	0.94 ± 0.09
Lac/Cr	0.3 ± 0.3	3.4 ± 1.1*	0.9 ± 0.6
LPS+HYPOXIA			
T2 (ms)	72.5 ± 3.6	60.7 ± 2.3*	70.5 ± 2.1
T2* (ms)	35.3 ± 9.2†	23.2 ± 3.8* †	31.9 ± 3.2†
Dav ($1x10^{-3}$ mm^2/s)	0.99 ± 0.03	0.88 ± 0.06*	0.96 ± 0.05
Lac/Cr	0.3 ± 0.5	3.9 ± 1.3*	0.6 ± 0.4

**Table 2. Results (mean ± SD) ***indicates significant change vs baseline during hypoxia ($p<0.05$) † indicates significant intergroup difference ($p<0.05$)

Discussion and Conclusions The observed decrease in global ADC suggests that despite anaerobic glycolysis, indicated by the large increase in Lac peak-area ratios, this hypoxic insult results in impaired cellular energy generation. However, both the size of ADC fall (only 10-15%) and lack of histological cell death[1] suggest that the extent of energy failure following hypoxia alone is limited. Pretreatment with LPS did not appear to increase the effect of hypoxia on cellular energetics (the magnitude of ADC and lactate/Cr change was similar in both groups). The decrease in T_2* during hypoxia is likely to be due to a reduction in blood oxygenation as well as a compensatory increase in cerebral blood volume. The lower baseline values for T_2* observed in the endotoxin group are consistent with LPS increasing cerebral blood volume. This could blunt the vasodilatory response to subsequent hypoxia and account for the attenuated fall in T2* observed in this group. Although it is possible that these haemodynamic perturbations may contribute to the increased cell death observed with LPS pre-treatment, the absence of any effect of LPS on the metabolic response to hypoxia suggests that this synergistic effect is more likely to be caused by an endotoxin related upregulation of cellular inflammatory mechanisms.

References

1. Wang et al; Society for Neuroscience, 2004
2. Provencher SW; NMR Biomed. 2001 Jun;14(4):260-4.

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