Remote Ischaemic Post Conditioning is Neuroprotective in White Matter in a Piglet Model of Perinatal Asphyxia: an MRS and Immunohistochemistry Study

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Introduction: Neonatal encephalopathy (NE), subsequent to perinatal hypoxia-ischaemia (HI), is associated with high mortality and morbidity rates worldwide. Although successful treatment strategies such as therapeutic hypothermia[1] are in use in the developed world in intensive care settings, around 40% of treated infants still have adverse outcomes. There is an unmet need to develop novel, non-invasive approaches, which can be used alone or in combination with hypothermia to augment neuroprotection. One such simple protective phenomenon is remote ischaemic postconditioning (IPostC). This refers to the protective effect elicited in one organ from the application in another organ or tissue of several brief intermittent episodes of ischaemia at the onset of reperfusion. The aim of this study was to assess whether hind limb remote IPostC at resuscitation after transient hypoxia-ischaemia (HI) is neuroprotective based on 1H and 31P MRS cerebral biomarkers and immunohistochemistry in a piglet model of perinatal asphyxia.

Methods: Experiments were performed under UK Home Office guidelines. Sixteen healthy piglets (aged <48hr) were anaesthetised and physiologically monitored with intensive life support. Transient cerebral HI was induced by reducing the inspired oxygen fraction to 6% and inflating bilateral carotid artery occluders as previously described[2]. An external remote IPostC device designed to occlude the femoral arteries was fitted in all cases; at the end of transient HI subjects were randomised to 2 groups: i) remote IPostC:4 x 10 min cycles of lower limb ischaemia/10 min reperfusion, via remotely controlled inflation of the femoral artery occluders within the bore of the MRI(n = 8); ii) Controls: no inflation of the femoral artery occluders (n=8). Laser Doppler and the disappearance of hind limb oxygen saturation confirmed bilateral lower limb ischaemia during the femoral artery occlusion cycles. ^{1}H and ^{31}P MRS were acquired at baseline and at 24 hrs and 48hrs after HI using a 9.4 Tesla Agilent spectrometer with ^{31}P data also acquired sequentially during HI and for 1 hr afterwards. ^{1}H MRS data were collected from voxels located in the dorsal right subcortical white matter (WM voxel, 8x8x15mm) and in the deep grey matter centred on both lateral thalami (DGM voxel, 15x15x10mm), see figure 1, using a LASER acquisition (TR = 5000ms, TE =288 ms, 128 averages). Whole-brain ^{31}P MRS data were acquired using a single-pulse acquisition (TR = 10 s, 60 averages; 6 averages during HI). Spectra were analysed using AMARES[3] as implemented in the jMRUI software[4] and the following metabolite ratios were calculated: Lactate (Lac) / N-acetyl aspartate (NAA) from ^{1}H MRS data and nucleotide triphosphate (NTP)/epp(epp = exchangeable phosphate pool = Inorganic phosphate + Phosphocreatine + 2γ-NTP + β-NTP) from ^{31}P MRS data.Insult severity was estimated by calculating the time integral of the change in NTP/epp during HI and the first 60 min of resuscitation, as described previously[2]. Grouped data are given as mean (standard deviation). MRS data were analysed using a mixed effects linear regression model. For histology, the brain was perfusion fixed and paraffin embedded and stained for nuclear DNA fragmentation using histochemistry with TUNEL.

Figure 1: Voxel positions

Results: There were no physiological differences between groups (Table 1). One subject in the remote IPostC group did not have ^{1}H MRS because of technical problems. One subject in the control group was terminated priorto 48 h. Remote IPostC reduced TUNEL postive cell number in the internal capsule (figure 2).Table 2 shows the results from the mixed effects model. Predicted values from the model for WM Lac/Naa are significantly higher at 48 hrs in Controls than in remote IPostC. Predicted values for NTP/epp are significantly lower in Controls than in remote IPostC. Figure 3 shows the predictions from the model for WM Lac/Naa and NTP/epp adjusted for time and treatment group. DGM Lac/Naa showed no significant differences between Controls and remote IPostC.

Mean (SD)	Controls	Remote IPostC
Age (h)	35.8 (10.9)	33.0 (10.0)
Bodyweight (kg)	1.80 (0.22)	1.83 (0.21)
Duration of HI (min)	20.5 (2.6)	20.6 (1.9)
Insult severity (x 10^{-2} min)	9.6 (3.3)	9.1 (3.10)

Table 1: Physiology

	Estimated difference in WM Lac/Naa (remote IPostC vs Controls) [95 % confidence interval]	p-value	Estimated difference in NTP/epp (remote IPostC vs Controls) [95 % confidence interval]	p-value
Baseline	0.03 [-0.24, 0.30]	NS	-0.003 [-0.025, 0.020]	NS
24 hrs post HI	0.36 [-0.33, 1.05]	NS	-0.010 [-0.039, 0.018]	NS
48 hrs post HI	1.83 [0.55, 3.11]	**< 0.01**	-0.052 [-0.102, -0.003]	**< 0.05**

Table 2: Predicted values from mixed effects model, difference between groups

Discussion: Remote IPostC was neuroprotective in the cerebral WM based on reduced WM Lac/NAA at 48h and reduced TUNEL positive cells in WM. Whole brain 31P MRS NTP/epp was preserved with remote IPostC. The protective mechanisms of remote IPostC include the interorgan transfer of protective factors (opiods, bradykinin, adenosine), receptor stimulation and activation of prosurvival pathways that interact to convey the IPostC stimulus from cell surface to mitochondria where many of the prosurvival and death pathways converge[2,3]. Remote IPostC may be a feasible neuroprotective intervention in NE; future studies will assess the duration of the therapeutic window for remote IPostC and whether remote IPostC augments hypothermic neuroprotection.

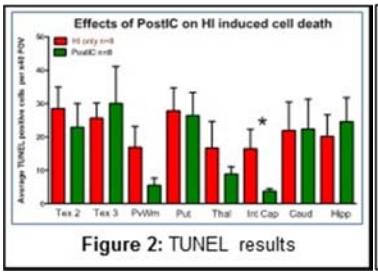

Figure 2: TUNEL results

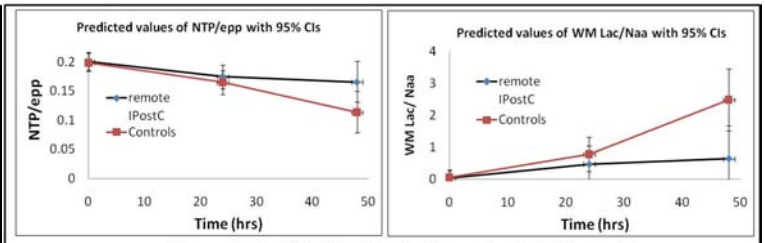

Figure 3: Predicted MRS ratios from mixed effects model

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