The Relationship between Impaired Cerebral Energy Metabolism and Apoptosis in the Cingulate Gyrus of Newborn Piglets following Transient Hypoxia-Ischaemia.

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Studies of newborn infants and piglets using 31P magnetic resonance spectroscopy (MRS) have demonstrated that hypoxia-ischaemia (HI) leads to biphasic changes in cerebral energy metabolism [1, 2]. A transient depletion of high energy phosphates is seen during the insult (measured by MRS as a fall in the [nucleoside triphosphate]: [exchangeable phosphate pool] ratio (NTP/EPP), which relates to cerebral ATP concentrations). This can return to normal after resuscitation, but is followed by a second phase of impaired energy metabolism beginning some 8 to 12 hours later.

The mechanism of delayed cerebral injury is unknown, although the kinetics of cell death are consistent with an HI-triggered physiological process such as apoptosis. In the present study we have examined the relationship between impaired energy metabolism during and following HI, and the fraction of cells undergoing apoptosis in the cingulate gyrus of newborn piglets. Sixteen piglets, all less than 24 hours old, were anaesthetized and ventilated while continuous 31P spectra were collected. Ten of these were subjected to bilateral carotid artery occlusion while the inspired oxygen fraction was reduced until NTP levels fell to less than 30% of baseline. The animals were then resuscitated and maintained for up to 48 hours. The remaining six animals underwent sham surgery as a control.

Immediately following sacrifice, brains were perfusion-fixed using 1% paraformaldehyde and coronal paraffin embedded sections (5 μm) were examined after staining with haematoxylin and eosin (H and E). The number of cells with the morphological characteristics of apoptosis was then counted in the cingulate gyrus; those cells demonstrating cytoplasmic shrinkage and containing basophilic nuclei that were either condensed (pyknotic) or fragmented (karyorrhectic) were defined as apoptotic. Sequential sections were also examined for single stranded DNA breaks using a nick translation technique.

As shown in Figure 1, the proportion of apoptotic cells, determined either by morphology, using H and E, or ISEL was linearly related to the severity of the injury determined by the degree of secondary energy failure (the ratio PCr / Pi) following resuscitation (p < 0.01).

These findings are in accord with recent observations in a rat model of focal cerebral HI injury in which DNA laddering has also been reported to occur [3-5]. Moreover, since there is a also a positive correlation between the ratio PCr to Pi and clinical outcome in newborn infants suffering birth asphyxia [6], our results suggest that neurodevelopmental impairment resulting from hypoxic-ischaemic brain injury may arise in part from the excessive or inappropriate activation of apoptosis.

References

Figure 1: Relationship between the delayed impairment of cerebral energy metabolism and the proportion of cells undergoing apoptosis. Results are presented as either the percentage of cells showing pyknosis and peroxidase staining when examined by in situ end labelling (open circles) plotted against the ratio [phosphocreatine]: [inorganic phosphate] at the end of the study (final PCr / Pi).