Clinical Value of Magnetic Resonance and Near-Infrared Spectroscopy in Neonates

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There is no doubt that cerebral ultrasound has proved an extremely useful technique for the neonatologist, who is increasingly concerned not only with reducing perinatal mortality but also with improving the quality of neonatal survival. Over the last decade, we have learned a great deal about the pathogenesis, natural history, and prognosis of periventricular hemorrhage in preterm infants, which is ideally demonstrated by ultrasound. However, ultrasound is less satisfactory for the early detection of cerebral hypoxic-ischemic lesions which are now recognized as a major cause of severe neurodevelopmental problems in preterm survivors (1). The motivation to explore other technologies for investigating the newborn brain, such as magnetic resonance spectroscopy (MRS) and near-infrared spectroscopy (NIRS) stems from the inadequacy of conventional techniques for studying cerebral ischemic lesions. This applies not only to preterm infants, but also to full-term infants who suffer hypoxic-ischemic encephalopathy following severe birth asphyxia, because these infants also contribute considerably to the toll of perinatally acquired handicap in the community.

PHOSPHORUS-31 MAGNETIC RESONANCE SPECTROSCOPY ($^{31}$P MRS)

MRS has been used as a nondestructive technique of biochemical analysis since it was first separately described in 1946 by Bloch et al. (2) and Purcell et al. (3). It is only in the last two decades that the biological applications of MRS have become apparent. In 1973 Moon and Richards first studied suspensions of red blood cells (4), and studies of cells, tissues, and isolated organs soon followed. The technological innovations that permitted the introduction of larger and more powerful superconducting magnets into clinical practice allowed the study of human organs in vivo. Neonatal brain studies were reported in 1983 (5).

The technique of MRS relies on the magnetic properties of certain nuclei, such as phosphorus-31, that align in a strong uniform magnetic field. These nuclei, within the background magnetic field, emit a radiofrequency (RF) signal when stimulated at their particular resonance frequency. This signal has a frequency that is determined
by the molecular environment of the $^{31}$P nucleus, so different phosphorus-containing compounds can be separated on the basis of their very slightly different resonance frequencies (chemical shifts). The signal also has an amplitude which is determined by the number of phosphorus nuclei resonating at any one frequency, and can be used to determine the molar concentration of the different phosphorus-containing compounds (6).

The spectrum shown in Fig. 1A was obtained from a healthy 3-day-old infant. The spectrum was recorded over a period of about 5 min while the baby slept within the 1.9-tesla superconducting magnet of a Bruker Biospec spectrometer. Radiofrequency pulses of 275 μs duration at 3-sec intervals were transmitted from an RF coil beneath the side of the baby’s head, and the resulting sequence of MR signals was detected by a 6.5 cm receiver coil. This coil collected MR signals from a large volume of one cerebral hemisphere, and these raw signals were summed and Fourier transformed to provide a frequency spectrum. No sedation or medication is needed for MRS studies, which do not involve ionizing radiation. However, there are major logistic difficulties in studying sick, and especially ventilated, infants in a powerful magnetic field. If iatrogenic disasters are to be avoided, very strict attention to patient safety is needed.

Figure 1A shows the seven peaks seen in the spectrum from a normal brain. Peaks 1, 2, and 3 are predominantly beta, alpha, and gamma adenosine triphosphate (ATP), respectively, peak 5 is attributed to phospholipid bilayers and phosphodiesters (PDE), and peak 7 is phosphomonoester (PME), mainly phosphoethanolamine. The other two peaks are of more importance in assessing cerebral energy status. Peak 4 is phosphocreatine (PCr), and peak 6 (shaded) is inorganic orthophosphate (Pi). When cerebral oxygenation is significantly compromised ATP levels are maintained at the expense of the hydrolysis of PCr. This leads to a fall in PCr levels and a reciprocal rise in Pi, as shown in Fig. 1B from a severely asphyxiated infant, studied at 9 days of age. ATP levels are maintained in all but the most severely asphyxiated infants and ATP depletion is associated with a very poor prognosis.

The time sequence of changes following birth asphyxia is interesting, because spectra recorded in the first 12–24 h show that when a baby is stable following initial resuscitation, cerebral energy status (as reflected by the PCr/Pi ratio) is normal. There is a secondary deterioration of energy metabolism, reaching its nadir at 3–4 days, which is supportive evidence for two-phase cellular injury following brain ischemia (7).

MRS data, and particularly PCr/Pi ratios, have been shown to have major prognostic significance in newborn infants following episodes of cerebral ischemia. In a study of 61 such infants, Azzopardi et al. (8) showed that a PCr/Pi ratio below the 95% confidence limits for normal infants predicted death or severe neurological deficit with a sensitivity of only 68% but a specificity of 95% and a positive predictive value of 96%. In practice, therefore, an abnormal PCr/Pi ratio is highly predictive, but a normal PCr/Pi is not necessarily reassuring.

The relatively poor sensitivity of conventional MRS (which is especially evident
in those infants who subsequently develop pure motor deficits) may partly be related to the large volume of tissue interrogated by the surface coil. Signals from superficial tissues tend to dominate the "global spectrum" recorded in the classical MRS experiment, and areas of significant energy impairment may be missed, especially if they are deeper in the brain, for instance in periventricular white matter.

Recent work we have carried out in Oxford, in collaboration with Professor Radda’s department, has tried to overcome this deficiency by using an MRS technique called phase-modulated rotating frame imaging (PMRFI). PMRFI uses a linear gradient in the RF field to obtain depth-resolved MR spectra, which permits noninvasive biochemical analysis of sequential discs of cerebral tissue about 0.6–1 cm in depth (9). Figure 2, obtained with the help of Bheeshma Rajagopalan, Martin Blackledge, and Nick Bolas, shows three spectra from the same asphyxiated infant whose global spectrum is shown in Fig. 1B. The spectra are from increasing depth into brain tissue.

PMRFI has confirmed that energy impairment following asphyxia is greater at 1–1.5 cm below the brain surface than in more superficial regions, adding support to
the suggestion that subcortical white matter, especially the base of the sulci, is a zone of vascular watershed in the full-term brain (10). Follow-up studies of infants studied by both conventional MRS and PMRFI are at an early stage. Preliminary results show that depth-resolved data are no more predictive than global data in a population of asphyxiated full-term infants. Because damage to periventricular white matter is more common in preterm infants, it may be that PMRFI will have a particular role in assessment of ischemic damage of the preterm brain.

Future potential developments of MRS as a tool for the investigation of the newborn include the study of organs other than the brain, the sophistication of spatial localization techniques, and the study of nuclei other than phosphorus. PMRFI permits study of the liver and heart, because it facilitates the exclusion of MRS signals from overlying muscle. If problems of increased ambient noise and of signal-to-noise ratio can be overcome then combined imaging/spectroscopy systems will allow spectra to be obtained from specific anatomical regions of brain defined by using a cursor on the proton image. Of the alternative nuclei, the technique likely to be of most clinical value is high-resolution proton spectroscopy with water suppression, which has already been used to measure cerebral intracellular lactate concentrations.

MRS has proved a useful research tool for the noninvasive investigation of intracellular biochemistry, and has clinical potential as a prognostic tool. However, there are considerable logistic problems with the technique that preclude its widespread
MRS AND NIRS IN NEONATES

application as a method for general use in clinical neonatology. It is very labor intensive, time-consuming, and requires infants to be transported to the superconducting magnet. The study of sick and ventilator-dependent infants is difficult, and involves considerable patient disturbance and customized apparatus without ferrous metallic components. These limitations were the main stimulus toward the development of alternative technologies for the assessment of cerebral hypoxia-ischemia in neonates requiring intensive care. One such potentially important bedside technique is near-infrared spectroscopy (NIRS).

NEAR-INFRARED SPECTROSCOPY

Living tissues are easily penetrated by light in the near-infrared part of the spectrum (wavelength 700–1,000 nm) and this property has been exploited in near-infrared spectroscopy (NIRS). Three major biological pigments, or chromophores, have characteristic absorption spectra for near-infrared light. These are oxyhemoglobin, deoxyhemoglobin, and oxidized cytochrome aa$_3$, the terminal enzyme in the mitochondrial electron transport chain. If the absorption characteristics of these three compounds are known, then the attenuation of near-infrared light at three or more wavelengths during its passage through living tissues can be used to calculate the amount of each chromophore in the light path.

Although NIR light passes through brain tissue much more readily than visible light, the loss in intensity is still approximately one order of magnitude per centimeter of tissue. The development of clinically useful systems has therefore relied on the use of high-powered pulsed-laser diodes to produce NIR light at very specific wavelengths, and sensitive photomultipliers operating in photon-counting mode for the detection of the transmitted light. Current technology allows measurements to be made by transmission spectroscopy across heads of 8-cm width using an average power output of 16 mW, which is an order of magnitude below recommended exposure limits. Larger heads can be studied using reflectance spectroscopy, but quantitation of results is less straightforward.

NIRS was pioneered by Franz Jobsis in North Carolina, who first reported studies of animal brains, and a preliminary human experiment in 1977 (11). His NIR spectrometer, marketed as the NIROS-SCOPE, was used in the first study of neonatal brains to be published from Duke University in 1985, by Brazy, Jobsis, and others (12). They described the derivation from animal experiments of the algorithms used to generate the on-line signals corresponding to amounts of oxy- and deoxyhemoglobin and cytochrome aa$_3$ in the light path, as well as a derived value of tissue blood volume. The paper reports the use of this system to display these cerebral hemodynamic variables in real time at the bedside, and to observe the perturbations caused by changes in the infant’s ventilatory status. Quantitation was not attempted in this study.

Further longitudinal monitoring studies have been reported by the group working under Professor Osmund Reynolds and David Delpy at University College Hospital
FIG. 3. Block diagram of near-infrared spectroscopy system. (Reproduced from the manufacturer's specifications, with permission of Hamamatsu Photonics Ltd.)

(UCH) in London (13). The UCH group has used a spectrometer developed in cooperation with a Japanese firm, Hamamatsu Photonics, diagrammatically represented in Fig. 3 (14). Figure 4A shows the effects on cerebral hemodynamics of an episode of desaturation in a preterm infant, as demonstrated by NIRS. Figure 4B shows the rise in oxyhemoglobin and oxidized cytochrome aa \textsubscript{3} that occurred in the brain of the same infant in response to a rise in pCO\textsubscript{2}.

The UCH group has also developed quantitative analysis, by using NIRS measurements to derive absolute values for cerebral blood flow, cerebral blood volume, and CO\textsubscript{2} reactivity (15). These developments mean that NIRS can be used to derive comparable data from different infants, rather than just measure relative changes longitudinally in single patients.

Absolute quantitation depends on knowledge of the path length of photons between source and sensor, which is considerably complicated by the effect of scattering. A value of 4.3 times the head width has been calculated as the path length by mathematical modelling, and this has been confirmed by "time of flight" measurements using ultrashort pulses of light and a "streak camera" (16).

Cerebral blood volume (CBV) in ml/100 g brain tissue can be calculated from
FIG. 4. A: NIRS measurements from a 27-week gestation, 1-day-old infant with no cerebral abnormality. ———, Oxyhemoglobin; ———, deoxyhemoglobin; - - - -, oxidized cytochrome aa₃. Transient decrease in arterial oxygen saturation [96% at a and c, 84% at b. B: Further NIRS measurements from the same infant as in A, during an increase in pCO₂ from 4.6 kPa to 5.7 kPa between a and b. (Data from E. O. R. Reynolds, J. S. Wyatt, D. Azzopardi, D. T. Delpy, E. B. Cady, M. Cope, S. Wray, Br Med Bull 1988;44:1052-75, with permission.]

measuring the effect on oxy- and deoxyhemoglobin of a small gradual change in arterial oxygen saturation. CBV has been noted to increase following birth asphyxia, presumably due to accumulation of cerebral vasodilators. CO₂ reactivity of the cerebral circulation, as measured by the change in cerebral blood volume resulting from a known change in arterial pCO₂, also falls following birth asphyxia, perhaps because of maximal cerebral arteriolar vasodilatation. Recently Edwards and the UCH group (17) have reported the use of NIRS to measure cerebral blood flow by an adaptation of the Fick principle. Oxygen is used as the tracer, and the input function is detected
by an adapted pulse oximeter on the ear. The arrival of the pulse of oxygen in the brain is detected by NIRS, and the values for cerebral blood flow obtained are consistent with values obtained using radioactive tracer methods.

These techniques have been used to show a prolonged and significant fall in cerebral blood flow following the administration of indomethacin to preterm infants for the medical closure of the patent ductus arteriosus. As well as monitoring the effect of other drugs, interventions, and diseases that may alter cerebral hemodynamics, NIRS may develop a diagnostic and prognostic role in the asphyxiated full-term infant. Cerebrovascular events in the first 24 h of life may be of major importance in the pathogenesis of the secondary energy failure demonstrated by MRS. NIRS may prove useful for the detection of a particularly high-risk group of infants and monitoring of new treatments in this period.

There are obvious attractions to bedside monitoring of cerebral oxygenation, and NIR spectrometers are now in commercial production. The NIR light is transmitted to and from the infant's head by fiberoptic cables attached to small "optodes" which are attached to each temporal region. There is relatively minor disturbance of the infant, but exclusion of ambient light is absolutely essential and the head has to be wrapped with a light-occluding cloth. Measurements are difficult in a struggling baby, and a considerable amount of "postprocessing" of the data on a separate microcomputer is currently necessary to obtain the derived values discussed above. NIRS is still basically a research tool. It will be interesting to see whether its clinical value is proven over the next few years, in which case further generations of spectrometers with more specific software should make NIRS more clinically accessible.

The advent of MRS and NIRS has added considerably to the range of available techniques for the objective assessment of the hypoxic-ischemic neonatal brain. NIRS measures cerebral oxygenation and by measuring oxidized cytochrome aa₃ also reflects intracellular oxygen availability. MRS measures intracellular concentrations of the high-energy phosphorus compounds ATP and PCr, which become depleted following severe hypoxia. The combination of the two should permit valuable noninvasive insights into the mechanisms of neuronal damage following cerebral hypoxia-ischemia. They may point the way to possible therapeutic maneuvers, and should also be useful tools for the objective assessment of the effect of such interventions.

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REFERENCES


DISCUSSION

Dr. Roloff: Those of us who do ECMO may wish to have this machine in our unit in order to have the method available for research on tolerance of hypoxic insults.

Dr. Hope: There are many things to look at, and we are starting with more traditional therapies. There are numerous questions still to be answered about the use of inotropes in the preterm infant, and about the degree of volume loading necessary to achieve cerebral perfusion. These are clinical problems that we face every day and I think we still largely work on prejudice. One of the interventions we perform about which there has been much concern is the use of indomethacin, which has been shown both on Doppler and more recently on NIRS to be associated with a considerable and sustained fall in cerebral blood flow, whether it is given by bolus or by continuous slow infusion. So there are many interventions to test including, perhaps, ECMO in the long term.

Dr. Roloff: Do you find that treatment methods we ordinarily consider as indicated to improve oxygenation may result in decreased tissue oxygenation while PO$_2$ or other accepted measures of oxygenation improve?

Dr. Hope: Certainly. Skin oxygenation, for example, may be a poor indicator of brain oxygenation. If we could demonstrate satisfactory cerebral oxygenation at lowish pO$_2$ values, maybe we could avoid unnecessary barotrauma in some preterm infants.

Dr. Holzgreve: Can the technique be used to identify placental infarction, particularly in the anterior wall, which is close to the surface of the maternal abdomen?
Dr. Hope: It is a major problem to look at tissues more than a few millimeters or maybe a centimeter deep. Light intensity falls exponentially with depth so the majority of the NIR signal will come from the maternal abdominal wall. People have looked at the placenta with magnetic resonance spectroscopy and this is feasible with an anterior placenta.

Dr. Rosén: Is there a possibility of using deep frozen specimens for magnetic resonance analysis?

Dr. Hope: It is increasingly realized that even the most sophisticated freeze-clamping results in degradation, especially of phosphocreatine. Especially in larger organs, where freezing is not instantaneous, freeze clamping is probably rather less accurate than in vivo MRS.

Dr. Merchant: Are there any data, either with NMR or infrared, to assess the effectiveness of therapy? Have any infrared studies been done after therapy for asphyxia in experimental animals?

Dr. Hope: Some studies have been done but they are very difficult. At University College Hospital we looked at the effect of mannitol infusions in asphyxiated infants in whom ultrasound examination suggested cerebral edema. There was no immediate effect on cerebral energy metabolism in the 30 min following a standard bolus of mannitol. Experimental studies involving the survival and recovery of severely asphyxiated animals are very difficult and in Oxford we are currently limited in our ability to study neonates sequentially because our magnet is in a different building.

Dr. Merchant: If such data were available one would have extremely strong evidence to show which forms of therapy are most effective in cerebral edema and asphyxia.

Dr. Hope: There are exciting prospects in terms of therapy, particularly the use of calcium entry blockers and antagonists of excitatory neurotransmitters.

Dr. Caccamo: How long does an NMR examination last? Is an anesthetic necessary?

Dr. Hope: No. Most neonates lie still after a feed. The global spectrum takes 5–10 min and the full PMFR “image” takes about 20–30 min.

Dr. Dawes: That length of time bothers me because you can expect a change of energy distribution in the brain during sleep cycles, particularly in the brain stem nuclei. Is there a possibility of shorter measurement periods?

Dr. Hope: The signal-to-noise ratio is dependent on field strength; a higher field strength improves sensitivity. However, there is the important issue of biological safety. The current recommended upper limit of field strength is 2.5 tesla, i.e., just above the value of 1.9 tesla that we are using. By the time one reaches 4 tesla and above, biological effects such as induced potentials in the field are more likely. If we can’t increase the magnet strength, then at least a few minutes of measurement are required to obtain acceptable signal-to-noise ratio to get the global spectrum, and longer to use any of the current techniques of spatial localization. I should like to ask whether you think the sort of energy failure we see is likely to occur during sleep cycles. Our technique tells us about severe brain injury but I should be surprised if energy metabolism was deranged and phosphocreatine depleted during sleep cycles.

Dr. Dawes: That is a perfectly good question and the answer is no. You are probably dealing with degradation of energy reserves far greater than normal.