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COMMENTARY

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) in Neonatal Brain Injury

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With the advances in reproductive medicine and neonatal intensive care, we are confronted with an increasing number of high-risk newborns that suffer from considerable neurologic morbidity which often is associated with lifelong handicaps. New diagnostic tools are therefore needed to assess brain development, to detect early brain injury and to monitor interventions aimed at minimizing or preventing irreversible brain injury. Magnetic resonance (MR) techniques are attractive for use in newborns because of their ability to provide detailed structural as well as metabolic information without the use of ionizing radiation. One of the essential contributors to the progress in noninvasive detection of tissue metabolism and *in vivo* biochemistry in recent years has been ^{1}H-magnetic resonance spectroscopy, which gives specific chemical information on the biochemistry of numerous intracellular metabolites. Neurochemistry has particularly benefited from this technique, thanks to the possibility of detecting cerebral metabolites *in vivo* in otherwise inaccessible tissue.

Magnetic resonance spectroscopy (MRS), like imaging uses the intrinsic magnetic properties of some atomic nuclei (*e.g.* ^{1}H, ^{31}P, ^{19}F, ^{13}C) having a nonnull spin number and magnetic moment. When a group of magnetically active nuclei are placed in a static magnetic field, a specific radiofrequency (RF) energy can be absorbed or emitted and can be detected. This resonant frequency, called Larmor frequency, depends on the nuclei of interest and the applied magnetic field. The most commonly used nucleus for clinical cerebral applications is ^{1}H proton, because it is the most abundant and strongest nucleus. More specifically, MRS takes advantage of the fact that the same nuclei in different molecules (*e.g.* water or triglyceride) or in a different part of a molecule have a slightly different resonance frequency, due to the different electron density, which shield the static magnetic field to varying degrees. This phenomenon is called chemical shift and is generally expressed in parts per million (ppm) and is by convention referenced to a specific material; for instance, water at 4.7 ppm is used for ^{1}H MRS. Thus different signals seen in the spectrum correspond to distinct molecules. Nevertheless some molecules (*e.g.* creatine and phosphocreatine) cannot be resolved *in vivo* with the magnetic field commonly used in clinical practice.

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^{1}H-MRS contains a wide array of interesting metabolites, all hidden in the spectrum underneath a large water resonance. By the strategy of using additional water suppression pulses the *in vivo* brain spectrum then reveals the following major metabolites: N-acetylaspartate (NAA); creatine+phosphocreatine (Cr); choline-containing compounds (Cho); myo-inositol (mI); glutamine and glutamate (Glx) and lactate (Lac). (see Fig. in paper Roelants-van Rijn *et al.* in this issue). For the above mentioned compounds the spread of the chemical shift (ppm range) is less than 10 ppm, which makes the ^{1}H spectra complex to analyze and quantify due to the high degree of overlapping resonances. A way to simplify the ^{1}H spectrum is to use long echo times. This has two effects: the disappearance of compounds with short echo times (*e.g.* macromolecules and lipids) which eliminates the spectrum baseline, and the elimination of certain compounds (*e.g.* mI, glx) which have multiple spectral lines due to interactions between neighboring ^{1}H nuclei, known as J-coupling. For lactate, this J-coupling results in a negative peak for TE = 135 ms and a positive signal at TE = 270 ms, for which these two long echo times are commonly used to reliably detect Lac with ^{1}H-MRS. The metabolites best measured at long echo times are NAA, Cr, Cho and Lac.

NAA, a free amino acid is present at the second highest concentration in the human CNS after glutamate. During early brain development the NAA concentration increases with regional differences, the thalamus expressing NAA early in development, with occipitoparietal and periventricular white matter later in development (1–7). It has been shown to be uniquely localized in neuronal tissue, neurons and axons of the adult brain, while during development it is also found in oligodendrocyte-type-2 astrocyte progenitors cells and immature oligodendrocytes (8) and is therefore an ideal indicator of intact central nervous tissue. Ratio's of NAA/Cho and NAA/Cr have therefore been used to assess cellular metabolic integrity in neonatal brain injury (9, 10). Studies using ^{1}H-MRS at a distance (>1–2 wk) to the hypoxic-ischemic event showed good correlation between reduced NAA ratios with adverse neurodevelopmental outcome (9–13) where as in early (acute stage) ^{1}H-MRS NAA ratio's do not correlate as well with outcome.

More recent studies have used absolute quantification of ^{1}H-MRS to define developmental changes in NAA concentra-

tion (1, 3, 5, 6, 14) and have confirmed the reduction of cerebral NAA in the chronic stage of severe hypoxic-ischemic perinatal brain injury (15, 16).

Neurons have a poor ability to regenerate, and thus a continuous provision of energy supply is essential for the function and integrity of the brain. Lactate occupies a special position in energy metabolism. Being an end-product of anaerobic glycolysis, the lactate concentration must rise whenever the glycolytic rate in a volume of tissue exceeds the tissue's capacity to catabolize lactate or export it to the bloodstream. This takes place in the event of hypoxia or hypoxia-ischemia. Early spectroscopy (<18h after event) and measurement of high Lac/Cr ratio in ^{1}H-MRS as well as low PCr/Pi ratio with ^{31}P-MRS correlated well with neurodevelopmental outcome at 1 y (17). This acute phase lactic acidosis is followed by persistently elevated lactate levels not associated with acidosis 1–2 wk after the event to several weeks after the hypoxic-ischemic event (11). This secondary lactate accumulation is accompanied by a decrease in NAA for which the ratio of Lac/NAA becomes a good marker of severity of hypoxic-ischemic encephalopathy in the subacute to chronic phase, as shown in the paper of Roelants-van Rijn *et al.* in this issue. Production of lactate by anaerobic metabolism is an unlikely explanation for this persistent Lac rise in damaged brain. In histology we find reactive astrogliosis during the subacute phase of brain hypoxia-ischemia (18). Glutamate uptake into astrocytes stimulates glycolysis within the astrocyte with production of lactate, that then is taken up by neurons and metabolized into energy (19, 20). The astrocyte therefore seems to be the cellular component responsible for the production of lactate in this late phase of hypoxia-ischemia.

Astrocytes play a variety of complex nutritive and supportive roles in relation to neuronal metabolic homeostasis. For example astrocytes take up glutamate and convert it to glutamine, this removal of glutamate from the extracellular space protects surrounding cells from excitotoxicity from glutamate. Glutamate and glutamine are both amino-acids that are measured in ^{1}H-MRS when using short echo times. During acute phase hypoxia-ischemia large amounts of glutamate, probably released from axonal injury and a major factor in posthypoxic cytotoxicity, are found in the extracellular space. Measurement of glutamate in *in vivo* spectroscopy though is difficult because of high spin-coupling. Increase of glutamate and glutamine combined have been observed by ^{1}H-MRS in chronic hepatic encephalopathy (21) and after hypoxic-ischemic injury in the pediatric patient (22). Roelants-van Rijn *et al.* in their study at a mean age after hypoxia-ischemia of 8 d were not able to show any significant changes measuring glutamate-glutamine ratio's, which can be due to the above mentioned technical problems or the already subacute phase of the hypoxic-ischemic event, when glutamate levels have normalized.

Osmoregulation is another major metabolic task fulfilled by astroglia. Osmolytes synthetized by astrocytes or present in astroglia include taurine, hypo-taurine and myo-inositol (23, 24). These metabolites can be measured only with short echo times, which require more robust acquisition scheme with reduced artifacts (*e.g.* water suppression, eddy curent). The quantitative evaluation of these metabolites further requires improved line fitting procedures and spectral analysis due to the overlapping resonances. Developmental changes of mI have been described with a decrease of mI during the first year of life and a marked reduction of mI in the first weeks after birth regardless of the gestational age at birth (5, 25). Recent data indicates that myo-inositol is increased after acute perinatal brain injury (26) which could indicate up-regulation of osmolytes after hypoxia-ischemia and acidosis. mI increase in the chronic stage perinatal white matter injury was also noted (27, 28) which might represent changes in cellular composition with an increase of astroglia. In the study of Roelants-van Rijn *et al.* in this issue mI/Cr ratios in the basal ganglia did not differentiate between good or bad outcome after perinatal hypoxia-ischemia and further studies are needed that take into account different brain regions and timing after insult to elucidate the role of mI.

There are other metabolites that become visible with short echo-time ^{1}H-MRS such as the macromolecules/lipids at 0.9ppm and 1.3ppm. These resonances show important changes in adult hypoxia-ischemia (29) and in experimental data on *in vitro* apoptosis (30). Evaluation of the significance of these metabolites in neonatal brain injury remains to be determined.

The quantification of metabolic information acquired from MRS bears several spectral and numerical difficulties due to 1) the overlay of resonances, 2) spin interaction (J-modulation), 3) variable metabolite relaxation times, 4) choice of line-fitting for numerical area-under-curve determination (peak-height, line-fit), 5) area ratios, such as presented in the article by Roelants-van Rijn *et al.* (lacking absolute concentrations) and 6) absolute quantification using external/internal standards.

The physiologist is usually interested in the intracellular concentration of a chemical species in a particular cell type. Human NMR measurement in single voxel MRS is an average (over the sensitive volume) of all tissue types. In the brain, therefore, we generally assess a combination of glial and neuronal cells with different extracellular space depending on how much white matter, gray matter, or cerebrospinal fluid the volume-of-interest contains. This can in part be overcome by multi-voxel techniques like chemical shift imaging (CSI) where spectra are obtained within a large 1-dimensional, 2-dimensional or even 3-dimensional volume-of-interest. The practical limitation of these methods is due to the very long acquisition times needed to complete the necessary number of minimum excitations per voxel inside the larger volume, and the rather poor definition of the small individual voxels. Also, only that proportion of a metabolite, which is mobile will yield an MRS-visible signal. Phospholipids, when incorporated in membranes or myelin, are not MR-visible. However if they are broken down or synthesized, then the phosphodiesters and monoesters products or the di- and triacyl groups become MRS-visible.

These methodological and technical limitations must be borne in mind in applying the results obtained from MR spectroscopy.

Despite the technical limitations mentioned above Magnetic Resonance Spectroscopy has opened up the possibility to study brain biochemistry and metabolism and indirectly characterize

brain tissue composition *in vivo*. It has added considerably in understanding specific pathophysiologic mechanisms in perinatal brain injury. ^{1}H-MRS has further become an important adjunct to diagnostic structural imaging that permits more accurate and earlier determination of prognosis after hypoxia-ischemia in the newborn.

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