14 MAGNETIC RESONANCE SPECTROSCOPY

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14.1 Introduction

Traumatic brain injury continues to be the major cause of death in individuals under 44 years of age (Chapter 1). In addition to the high degree of mortality, the morbidity associated with survivors of severe head injury imposes significant social and financial costs upon the community. Despite being such a major public health issue, the mechanisms that result in the irreversible tissue damage after brain trauma are still unclear. It has become increasingly accepted that irreversible tissue damage after brain trauma occurs through both primary and secondary means. Primary events are generally considered to be the mechanical changes to nervous tissue that occur at the time of insult, while secondary events are the physiological and biochemical changes that occur over time after the initiating insult (Chapter 4). The fact that the secondary events occur over hours to days after the trauma encourages attempts to prevent, or at least attenuate, the secondary injury cascade. Nonetheless, before preventative therapies can be introduced, there is a clear need to identify the factors involved in the secondary injury process, and a need to be able to identify whether a selected 'antifactor' is indeed having the desired effect on brain metabolism. While a number of techniques can be used in experimental studies to investigate metabolic events associated with trauma and pharmacological intervention, these are primarily invasive techniques that cannot be translated to a clinical setting. However, the recent application of non-invasive magnetic resonance spectroscopy (MRS) to experimental trauma studies has now resulted in MRS techniques being applied to metabolic studies of clinical head injury. The purpose of this chapter is to provide an overview of magnetic resonance spectroscopy and its utility in non-invasive metabolic studies. Recent clinical applications of MRS in head injury and how they correlate with previous experimental studies will be summarized, and newer magnetic resonance imaging (MRI) techniques for studies of brain metabolism and function will be briefly discussed.

14.2 Principles of magnetic resonance spectroscopy

It is not the purpose of the current review to describe in detail the basic principles of magnetic resonance, but rather to illustrate the non-invasive nature of magnetic resonance spectroscopy and its potential for studies of brain metabolism. For readers interested in a detailed description of magnetic resonance theory, there are a number of excellent articles available (Gadian, 1982; Iles, Stevens and Griffiths, 1982; Gordon, 1985).

Like all forms of spectroscopy, magnetic resonance relies on the excitement of a particle from a ground state to an excited state and the measurement of energy as the particle relaxes back into the ground state. In the case of MRS, the particle is the magnetic dipoles within the nuclei of the particular molecules of interest. When placed into a strong magnetic field, the magnetic dipoles assume either a low-energy state (where the dipoles align with the magnetic field) or a high-energy state (where the dipoles align against the magnetic field). Transitions between the two energy states can be induced by the application of an appropriate form of energy, which in this case is radio-frequency energy. Furthermore, different nuclei require particular frequencies of radio-frequency energy to induce the transition. Hence, proton nuclei, for example, require a different frequency of excitation from other nuclei such as phosphorus. Tuning in for a particular nucleus is therefore similar to tuning in a transistor radio to a particular radio frequency. Having tuned in to a nucleus of interest, applying a burst of radio-frequency energy will induce transitions in energy states in all molecules containing that nucleus. The transition from a low-energy state to a high-

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energy state and back again is known as **resonance**, and the whole process as **nuclear** (for the nuclear dipoles) **magnetic resonance**.

The relaxation of an excited nuclear dipole back into the low-energy state will emit energy in the form of radio frequency, which can be detected by a radiofrequency receiver coil. Again, the receiver coil must be tuned into the correct radio frequency to detect this release and it is not unusual that the one radiofrequency coil is used firstly as the transmitter and then as the receiver. The detected signal will decay with time as the dipole relaxes into the lower-energy state. By converting the amplitude *versus* time decay into an amplitude *versus* frequency plot (by Fourier transformation), the resulting MR spectrum will show a variable number of peaks whose amplitude is directly related to the number of nuclei undergoing resonance, and whose frequency identifies which nuclei are being excited. Although it excites one type of nucleus, nuclei in different chemical or ionic environments have their resonant frequency affected by that environment. The result is that slightly different frequencies are observed for each different environment experienced by the nuclei. For example, in a phosphorus MR spectrum, inorganic phosphate will have a slightly different frequency (chemical shift) from a phosphate molecule attached to an adenosine ring (adenosine monophosphate): the adenosine ring confers a different chemical environment upon the phosphate. Similarly, adenosine diphosphate will have a different chemical shift from adenosine monophosphate because of the different chemical environments being experienced by the phosphate groups, and so on. A typical phosphorus MRS spectrum of brain is shown in Figure 14.1. A number of phosphate metabolites can be recognized on the basis of previous work (Vink, 1993), including phosphomonoesters, inorganic phosphate, phosphocreatine, and the three phosphate groups of adenosine triphosphate, labeled as α, β and γ ATP.

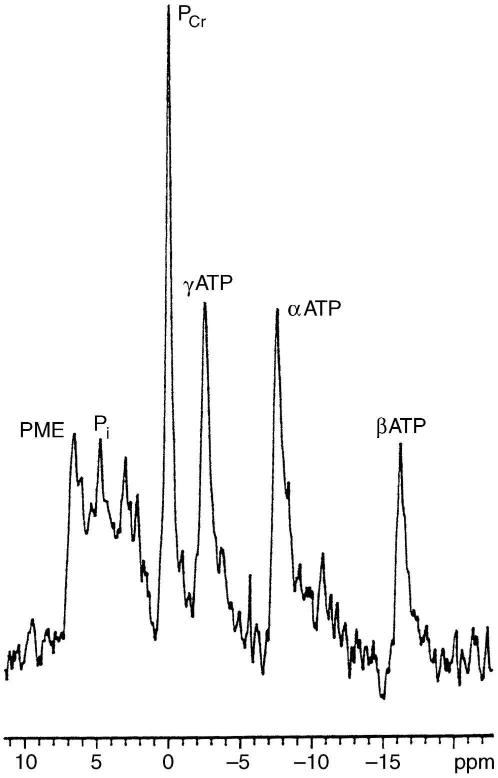

Figure 14.1 Typical phosphorus MRS spectrum of brain. Identifiable peaks include the phosphomonoesters (PME), inorganic phosphate (P_i), phosphocreatine (P_{Cr}) and the three phosphate groups of adenosine triphosphate (ATP).

Having considered how chemical environment affects the resonant frequency of different nuclei, it should be noted that ionic environment can also affect resonant frequencies. This property has proven very useful, particularly in phosphorus MRS where the effect of protons (H^+) on the inorganic phosphate chemical shift has been exploited to gain information on regional pH (Petroff *et al.*, 1985). In addition, a number of studies have used the chemical shift of the βATP peak to calculate values for intracellular free magnesium concentration (Gupta and Gupta, 1984).

14.3 MRS studies of neurotrauma

14.3.1 PHOSPHATES

The first applications of magnetic resonance spectroscopy to experimental traumatic brain injury were published in 1987 and used phosphorus MRS to focus on energy metabolism and pH after trauma (Ishige *et al.*, 1987; Vink *et al.*, 1987b). It wasn't long before the utility of MRS in clinical neurotrauma was recognized and the first study on human head injury was published in 1990 by Gennarelli and colleagues (Rango *et al.*, 1990). ***These authors found that in patients with a mean Glasgow Coma Score of 6.1, there were no significant changes in the phosphorus-containing metabolites between days 2 and 21 after injury when compared to normal controls. The lack of any apparent energy failure in this study was consistent with the animal studies published earlier. These results suggest that brain trauma, as opposed to brain infarction (Williams, Crockard and Gadian, 1989), does not cause overt energy failure as determined from the level of high-energy phosphate compounds in the MRS spectra.***

Nonetheless, subtle changes in phosphocreatine to inorganic phosphate (P_{Cr}/P_i) ratio have been reported in animal studies of trauma indicating an increased energy demand after trauma (Vink *et al.*, 1988a). These changes in P_{Cr}/P_i ratios have been correlated to the appearance of neurological deficits in traumatized animals (Vink *et al.*, 1988a), and similar correlations have been found clinically, at least in subdural hemorrhage (Yoshida *et al.*, 1994), birth asphyxia (Cady *et al.*, 1983; Younkin *et al.*, 1984) and seizures (Younkin *et al.*, 1986). Moreover, the studies of subdural hemorrhage (Yoshida *et al.*, 1994) have reported that evacuation of the hematoma results both in an improvement in P_{Cr}/P_i ratio and the disappearance of any hemiparesis. A closer inspection of P_{Cr}/P_i ratios in clinical trauma may thus be warranted, at least with respect to interventional strategies for subdural hematomas. ***A final point to be made about phosphate MRS of trauma is that animal studies have shown that imposition of a second stressor, such as hypoxia or hypotension, on a traumatized brain results in a significant decline in brain phosphate energy stores (Ishige et al., 1987, 1988). This finding suggests that the traumatized brain may be sensitized to secondary insults and that the subsequent appearance of a secondary event may initiate a considerable energetic perturbation.***

14.3.2 BRAIN pH

Phosphorus MRS spectra also contain information on brain pH. The phosphorus data from the original 1990 clinical study of human head injury (Rango *et al.*, 1990) demonstrated that intracellular pH was never in the acidotic range. In fact, there was a tendency toward alkalosis in the first days to weeks after trauma, which had resolved by 3 weeks. In a 1995 proton MRS study of pediatric head injury, Sutton *et al.* (1995) confirmed that diffuse axonal injury did not cause brain acidosis by demonstrating an absence of any increase in lactate concentration. Previous animal studies of trauma had shown that any acidosis after trauma was correlated with lactic acid accumulation (McIntosh *et al.*, 1987). Some cases of lactate accumulation were reported after clinical head injury but this accumulation was always associated with either localized contusion or cerebral infarction (Sutton *et al.*, 1995). Indeed, the appearance of acidotic regions in human brain has been proposed to be an indicator of focal ischemia (Brooke *et al.*, 1994). Moreover, their appearance was correlated to impaired consciousness and the normalization of pH within these infarcted regions over time coincided with an improvement in clinical condition. ***It is significant that these clinical trauma studies confirmed earlier MRS studies of experimental head injury (McIntosh et al., 1987) which demonstrated that any brain lactic acidosis after trauma was both mild and transient irrespective of injury severity (Figure 14.2). Indeed, significant acidosis only occurred under the most severe conditions, where mortality was 100% and global ischemia was suspected to be a contributing factor (McIntosh et al., 1987).***

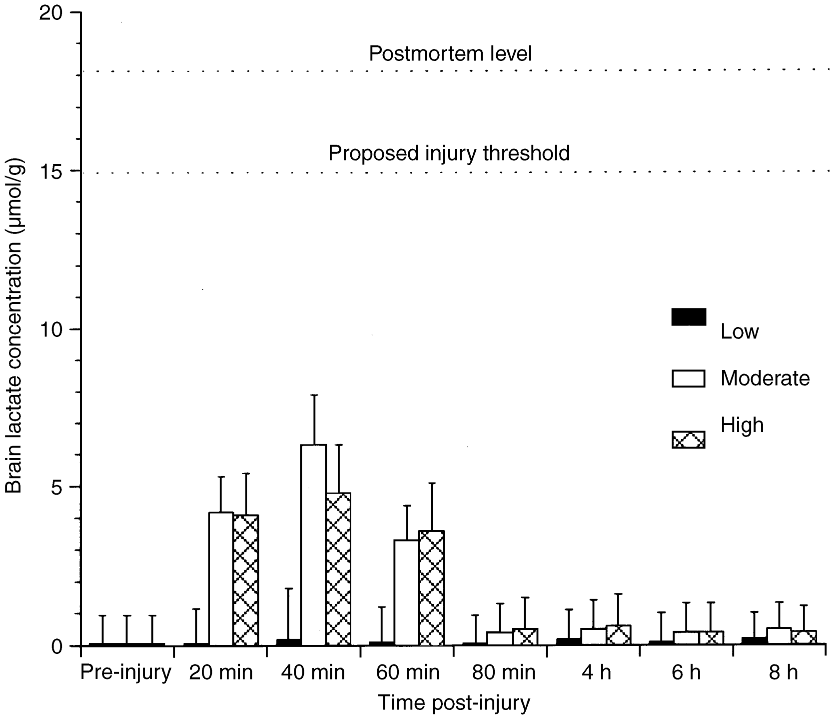

Figure 14.2 Temporal profile of lactate accumulation following low, moderate and high levels of experimental traumatic brain injury in rats. Lactate accumulation is transient and levels remain below what is considered to be the injury threshold. (Source: adapted from McIntosh *et al.*, 1987.)

Previous studies had shown that CSF lactate concentration may be a useful prognostic indicator following brain injury (DeSalles et al., 1986). However, MRS studies of experimental trauma confirmed that the injured brain may not be the source of the CSF lactate and that much of the CSF lactate may in fact originate from systemic sources (Inao et al., 1988). Although CSF lactate may still be a useful prognostic indicator after trauma, the conclusion from both the experimental and clinical studies described above is that therapies targeted at alkalinizing brain cells may only be of use in cases with suspected brain contusion or infarction. Moreover, while these conditions can be identified by MRI, it would be beneficial to use MRS to determine efficacy of the buffering agents.

14.3.3 FREE MAGNESIUM

The free magnesium ion is gaining increasing recognition as a critical factor in neuronal cell function. The finding that magnesium ions gate the n-methyl-D-aspartate class of glutamate receptors (Mayer, Westbrook and Guthrie, 1984) has rekindled earlier enthusiasm for magnesium as an important regulator of central nervous system function (Fishman, 1965). Indeed, magnesium has now been shown to play an active role in the regulation of calcium channels (Agus *et al.*, 1989), prostanoid synthesis (Nigam, Averdunk and Gunther, 1986), membrane peroxidation and free radical formation (Gunther *et al.*, 1994), energy metabolism (Ebel and Gunther, 1980), glycolysis (Garfinkel and Garfinkel, 1985), neurotransmitter release and binding (Rothman, 1983), cerebral vascular tone (Altura *et al.*, 1984), protein synthesis and DNA transcription (Terasaki and Rubin, 1985), among others. It is therefore clear that potential changes in magnesium ion concentration may have far-reaching consequences in terms of metabolic events and recovery after brain injury. The difficulty in demonstrating a role for magnesium in secondary injury after trauma has been that, unlike calcium, measurement of free magnesium concentration has been technically difficult and the available techniques largely inadequate. The recent development of phosphorus MRS techniques for the determination of intracellular free magnesium concentration has since permitted the reliable analysis of free magnesium concentration prior to and following a traumatic event.

The first application of phosphorus MRS to measurement of free magnesium concentration after experimental trauma appeared in 1987 (Vink *et al.*, 1987a) and was quickly followed by a succession of papers characterizing the potential role that magnesium decline after trauma may have in the secondary injury process (Vink *et al.*, 1988b; Vink and McIntosh, 1990; Vink, Faden and McIntosh, 1988; Vink, Golding and Headrick, 1994; Vink, Portoghese and Faden, 1991). ***In summary, these studies demonstrated that brain intracellular free magnesium concentration declines after trauma by as much as 60%. The significance of this may not be readily apparent to those unfamiliar with magnesium's role in biochemical and physiological processes, so Figure 14.3 shows the data transformed to a logarithmic scale and plotted adjacent to a more familiar parameter, pH.*** Free magnesium concentration typically changes by 0.25 units. If brain pH changed by 0.25 units (from 7.10 to 6.85), this would undoubtedly cause considerable concern! As opposed to the highly significant decline in free magnesium concentration, brain pH does in fact not change significantly after trauma.

Subsequent experimental trauma studies have demonstrated that pharmacological agents with neuroprotective properties in terms of outcome all improved free magnesium homeostasis (Vink, 1993). Notably, those agents that improved free magnesium homeostasis the most also had the greatest improvement in neurological motor scores. Recently, Smith and colleagues (1993) also demonstrated that improvement in magnesium homeostasis after trauma not only resulted in better neurological motor outcome after trauma but also significantly improved cognitive performance. Although the mechanism of action by which magnesium improves post-traumatic outcome is unknown, the fact that magnesium is a critical regulatory cation in such a large number of cellular functions (see above) dictates that any alteration in free ion concentration may have wide-ranging consequences.

Of course, the observation that magnesium declines following experimental trauma may not be apparent in clinical head injury. However, a recent study presented at the Third International Neurotrauma Symposium in Toronto (Lenkinski *et al.*, 1995) suggests that decline in free magnesium concentration is also typical of clinical trauma. This group reported that free magnesium concentration declines by approximately 50% and that this declines persists for at least 1 week. These observations have led to the recently commenced clinical trial using magnesium as a post-traumatic therapy to attenuate secondary damage (Lenkinski *et al.*, 1995).

14.3.4 PROTON MRS

With the exception of Sutton's recent publication on pediatric head injury (Sutton *et al.*, 1995), there have been very few reports concerning applications of proton MRS to clinical trauma. Nonetheless, the results from proton MRS studies in clinical ischemia

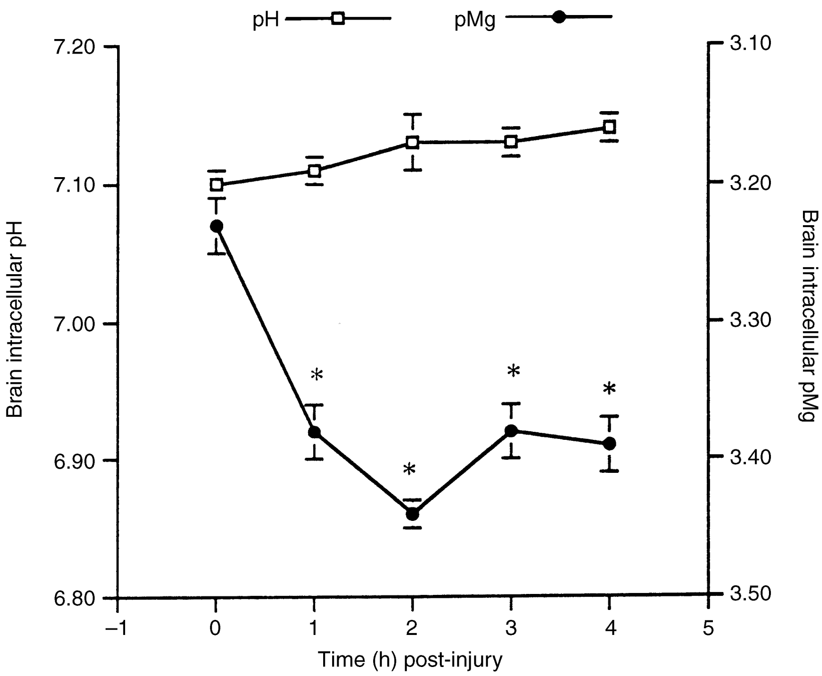

Figure 14.3 Intracellular pMg and pH levels following moderate traumatic brain injury. Intracellular pH typically does not change significantly after trauma whereas highly significant declines in free magnesium concentration occur immediately after trauma and persist for up to 1 week after injury. * = $p < 0.05$ from preinjury by ANOVA.

and neonatal development demonstrate potential applications to neurotrauma that are worthy of discussion. Of particular interest is the apparent correlation between n-acetyl aspartate (NAA) levels and degree of neuronal injury. N-acetyl aspartate is an intracellular intermediate metabolic product, required for neurotransmitter synthesis, which is present in large amounts in normal functioning mature neurons only. Its easily detected proton MR spectral peak has been shown in numerous studies to closely correlate with neuronal integrity and function and its loss or attenuation is an important *in vivo* indicator of acute or chronic neuronal depletion or destruction. ***A number of studies at the experimental and clinical level have now reported a decline in NAA after ischemic injury to the brain. Moreover, while phosphorus ratios derived from phosphorus MRS spectra normalize over time following the injury, NAA concentration as assessed from proton MRS spectra remains depressed (Peden et al., 1990; Houkin et al., 1993).*** Indeed, the NAA-to-creatine and NAA-to-choline ratios in the proton MRS spectra have been correlated to neurodevelopmental outcome in infants born with signs of hypoxic–ischemic encephalopathy (Peden *et al.*, 1993).

Declines in NAA concentration have also been found in experimental trauma (Lenkinski, unpublished results; Vink, unpublished results) and it remains to be determined whether such changes are observed in clinical head injury and whether any changes in NAA can be used for prognostic purposes.

14.4 MRI studies of brain function

Most clinicians and basic scientists should be familiar with the use of proton magnetic resonance as it is used in MRI. Simply stated, MRI uses the concentration of water to provide a spatial representation of water distribution throughout the tissues (Zimmerman *et al.*, 1986). Because of the difference in water concentration across various tissues, there is a 14% contrast available. Moreover, wherever water accumulates (as in edema, tumors or hemorrhage) the high concentration is easily detected by MRI. Recent technological developments in magnetic resonance now permit information about brain function, as opposed to brain anatomy, to be gained by such imaging techniques. By exciting water molecules using strong magnetic field gradients and obtaining images in a very short time, the diffusion and perfusion of those excited molecules in and out of a chosen plane can be monitored over time. Hence changes in blood flow and volume, increased blood oxygenation, intracellular accumulation of water and extracellular accumulation of water can all be visualized using these functional-type MRI techniques (for reviews, see Cohen and Bookheimer, 1994; Hossmann and Hoehnberlage, 1995). While few applications of these techniques to trauma have been reported to date, the potential is enormous.

14.4.1 APPLICATIONS TO TRAUMA

One of the first applications of MRI to gain functional information after experimental trauma was in studies of edema (Hanstock *et al.*, 1994). The discrimination of extracellular water accumulation (as in vasogenic edema) from intracellular accumulation (as in cytotoxic edema) can be accomplished by a technique known as **diffusion-weighted imaging**. In essence, this technique results in an image whose intensity is largely determined by the distance excited water molecules can diffuse over time. The shorter the distance a water molecule can diffuse the brighter the image intensity (e.g. intracellular water is restricted to within the boundaries of a cell membrane). In contrast, the greater the diffusion distance of water (e.g. interstitial water), the lower the image intensity. ***As opposed to studies of brain ischemia (Moseley et al., 1990), diffusion-weighted MRI studies of experimental traumatic brain injury demonstrated that early water accumulation following a traumatic event is largely vasogenic in nature (Hanstock et al., 1994), at least for the first 4 hours after the traumatic event.*** This observation supported the notion that there was no overt energy failure following trauma that might precipitate the development of cytotoxic edema. ***Rather, the brief opening of the blood–brain barrier after experimental trauma permits the accumulation of proteins in the brain extracellular space and the subsequent accumulation of water. In contrast, superimposition of a second insult such as hypotension or hypoxia on brain trauma results in profound cytotoxic edema (Ito et al., 1996) consistent with the previously proposed notion that combined insults leads to ischemia with associated energy failure (Ishige et al., 1987, 1988).*** Interestingly, Marmarou and colleagues (Ito *et al.*, 1995) also demonstrated that the amount of water accumulation in the extracellular space following trauma alone declines between 4 hours and 24 hours, implying that the extracellular space is getting smaller. ***Various factors may account for this reduction in extracellular volume, including development of cytotoxic edema and/or astrocyte swelling. Whatever the cause of the change with time, these results re-emphasize that trauma has a clear secondary component that develops over time.***

Such secondary injury, at least with respect to development of diffuse axonal injury, has also been confirmed using **magnetization transfer imaging** (Smith *et al.*, 1995). These authors were able to demonstrate that magnetization transfer imaging was more sensitive than conventional imaging techniques in detecting the presence of diffuse axonal injury. The axonal injury was detected at 3 days after injury despite no such indication on conventional MR images. Because of the significance of diffuse axonal injury in the development of severe injury, the early identification of such injury may prove to be of prognostic significance. ***Both diffusion-weighted imaging and magnetization transfer imaging have thus been reported to be more sensitive than any other technique in the early detection of edema and diffuse axonal injury, respectively.***

14.4.2 POTENTIAL FUTURE APPLICATIONS TO TRAUMA

Two further functional MRI techniques show particular promise for application to studies of traumatic brain injury. The first is perfusion imaging, used to measure blood flow. Cerebral blood flow has usually been determined by measuring the washout of an exogenously added tracer. ***Perfusion imaging works on a similar principle except that the 'tracer' is MR excited water in the vasculature ('arterial spin labeling') and washout is the signal decay in the region of interest (Detre et al., 1994). Consequently, estimates of cerebral blood flow and volume can be made non-invasively and repeatedly without the use of exogenous tracers.*** The technique has been successfully developed for use in human brain where changes in blood flow have been detected under conditions of hyperventilation or breath-holding (Roberts *et al.*, 1994). More recently, perfusion imaging has been nominated as a prognostic tool for predicting clinical outcome following ischemic stroke (Warach, Dashe and Edelman, 1996). Indeed, this early evidence suggests that the technique will be a valuable tool in distinguishing reversible from irreversible tissue injury. In trauma studies, the technique will be extremely useful in the determination of blood flow values associated with the purported 'ischemic threshold' that may or may not be similar to values obtained in ischemia studies.

The second technique that shows promise for application to trauma is what is known literally as functional MRI. The technique takes advantage of the effects of blood oxygenation on MR signal decay. ***The lower the oxygen concentration, the higher the concentration of deoxyhemoglobin and the more rapidly MR signal intensity decays. Thus, an increase in oxygen concentration in venous blood (due to increased flow relative to tissue oxygen consumption) results in an increased intensity in the MR image (Cohen and Bookheimer, 1994).*** Following the initial development in animal studies, functional MRI has been used extensively to demonstrate increased human functional brain activation in response to a specified task (Shulman *et al.*, 1993). In more recent clinical studies, the technique has been useful for mapping brain functional deficits following stroke (Sorensen *et al*., 1995) and in demonstrating cortical reorganization following focal perinatal brain damage (Cao *et al*., 1994). With respect to trauma, the technique has the potential to be used to map functional deficits as well as being a useful technique to predict functional outcome and to 'chart' the recovery of function in the injured brain during recovery from severe head injury.

14.5 Conclusion

There seems no doubt that magnetic resonance spectroscopy has made a significant contribution to the understanding of pathophysiological processes following experimental traumatic injury to the brain. Many of the early observations made in animals are now being confirmed in a clinical setting and a number of therapeutic strategies are being examined on the basis of these MRS findings. Nonetheless, despite the advances made in understanding the mechanisms of injury, it remains unclear whether MRS may develop into a useful prognostic indicator of outcome. Recent reports of both proton MRS and functional MRI studies suggest that the magnetic resonance techniques may indeed be useful in this regard. However, it remains to be determined whether the information gained from routine clinical MR evaluation justifies the expense of higher field magnets and the associated risks to the patient in such a high magnetic field environment.

14.6 References

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