Applications of magnetic resonance spectroscopy to chronic liver disease

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ABSTRACT – *In vivo* magnetic resonance spectroscopy (MRS) provides a non-invasive 'window' on biochemical processes within the body. The technique is currently a research tool, but new developments in whole-body magnetic resonance imaging (MRI) may provide a role for clinical MRS in obtaining functional information at the end of a standard MRI examination. Applications of hepatic and cerebral MRS to chronic liver disease and its associated complications are specifically considered in this article. Changes in phosphorus-31 MRS with the underlying functional severity of the cirrhotic liver are discussed. These reflect increased turnover of cell membranes as the liver attempts to regenerate. Patterns of spectral abnormality in the transplanted liver are described, and also the potential use of the technique to assess the viability of the stored donor liver in the time period between organ harvesting and implantation in the transplant recipient. Metabolite abnormalities in the brain are defined in hepatic encephalopathy and in patients infected with the hepatitis C virus who have minimal hepatic inflammation. Future trends are considered: for example, the use of MRS as a non-invasive tool to assess the effectiveness of liver-directed gene therapy.

Nuclear magnetic resonance background

The nuclear magnetic resonance (NMR) phenomenon was first demonstrated experimentally in 1946[1]. This technique has been widely used by physicists and chemists ever since. The biomedical applications of NMR are twofold (Fig 1):

- magnetic resonance imaging (MRI)
- magnetic resonance spectroscopy (MRS).

The applications of MRS as a research tool are extremely diverse, encompassing studies on isolated cells, body fluids and perfused organs at high magnetic field strengths in an experimental, laboratory-based setting and also *in vivo* studies using clinical MR systems[2].

In vivo clinical MRS has been used to study the metabolism of well-defined regions of the human body for the last 15 years, affording a non-invasive 'metabolic window' on a wide range of biochemical processes in the body, including the composition and function of human organs *in vivo*[3]. Clinical MRS developments have exploited many of the advances in MRI, such as the higher magnetic field strengths now used (typically 1.5–2.0 T) and the use of magnetic field gradients. The sensitivity and spatial resolution of MRS is a limiting factor *in vivo*, but parallel utilisation of *in vitro* NMR spectroscopy of tissue extracts, body fluids and cell lines at much higher magnetic field strengths (typically 11.7–14.1 T) allows more definitive interpretation of the *in vivo* data[3]. Some MR resonances are composite *in vivo*, but separation of all peaks can be achieved *in vitro* (Fig 2). By way of comparison, the strength of the earth's magnetic field is 0.3–0.7 x 10^{-4} T.

The radiofrequency transmitter/receiver system in clinical MRI scanners is generally tuned to hydrogen (^{1}H) nuclei, optimising the production of proton images (Fig 1). However, dedicated MRS systems

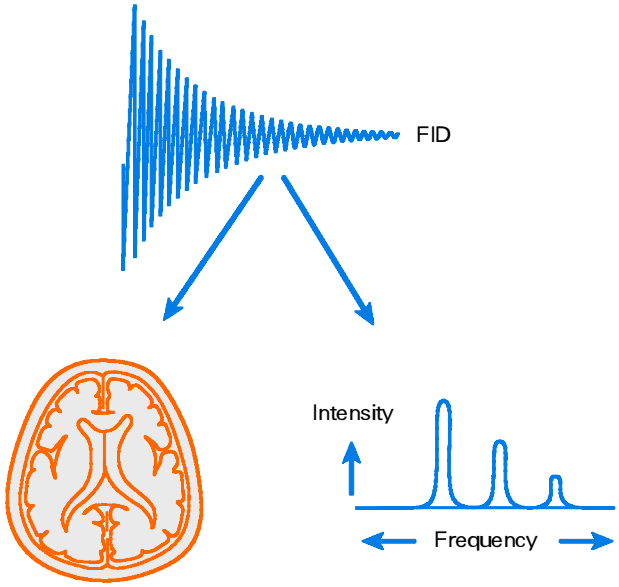

Fig 1. Principles of magnetic resonance.
The magnetic resonance (MR) signal or free induction decay (FID) may be converted by the mathematical process of Fourier transformation to form anatomical information (MR imaging) or localised biochemical information (MR spectroscopy).

have multinuclear facilities that allow studies on various NMR sensitive nuclei, particularly phosphorus-31 (^{31}P), as well as carbon-13 (^{13}C) and proton (^{1}H).

Spectral information

Analysis of the *in vivo* MR spectrum can allow non-invasive assessment of tissue metabolite concentrations in health and disease. Metabolite changes can be followed over time with sequential examinations. However, only compounds present in millimolar concentrations are detectable with *in vivo* MRS. The intensity of each metabolite signal is related to its concentration, but in practice absolute quantification is difficult to achieve. Many *in vivo* MRS studies therefore report results in terms of metabolite ratios[3].

In vivo ^{31}P magnetic resonance spectroscopy. The ^{31}P MR spectrum provides an assessment of energy status, membrane turnover and glycolytic/gluconeogenic intermediates[3]. A typical ^{31}P MR spectrum of the liver, obtained at the magnetic field strengths used for human clinical studies, contains six resonances (Fig 2). Phospholipid cell membrane precursors (including phosphocholine (PC) and phosphoethanolamine (PE)), adenosine monophosphate and glycolytic intermediates, such as glucose-6-phosphate, contribute to the phosphomonoester (PME) peak. Phospholipid cell membrane degradation products (including glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE)) and endoplasmic reticulum are the main components of the phosphodiester (PDE) peak. Information on tissue bioenergetics can be obtained from the inorganic phosphate (Pi) and the three nucleotide triphosphate (NTP) resonances[3]. The NTP resonances are not only composed of adenosine triphosphate (ATP) but are also contributed to in part by uridine, guanosine, inosine and cytosine triphosphates. Measurement of intracellular pH can be calculated from the relative chemical shift of the Pi resonance from a reference resonance[3]. Similar information can be obtained from *in vivo* cerebral MRS, although there is an additional large resonance from the high energy phosphate source, phosphocreatine (PCr).

In vivo ^{13}C and ^{1}H magnetic resonance spectroscopy. Data from ^{13}C are more difficult to obtain *in vivo*, mainly because this nucleus has a natural abundance of only 1.1% and the most abundant carbon isotope (^{12}C) is NMR insensitive. Despite this, signals from glycogen and lipids can be measured using natural abundance ^{13}C MRS, and quantitative measurements made[3]. However, most studies augment the NMR signal by administered non-radioactive ^{13}C-labelled tracers, for example ^{13}C-glucose, to follow metabolic pathways *in vivo*.

It is not easy to obtain ^{1}H MRS information from the liver *in vivo*. The high water and fat content results in MR signals that dominate the hepatic spectrum and are difficult to suppress adequately because of respiratory motion. However, the water signal can be suppressed in the brain, and a typical cerebral ^{1}H MR spectrum includes choline (Cho), creatine (Cr), myo-inositol and N-acetylaspartate (NAA) resonances. Myoinositol is a major intracellular osmolyte, whereas NAA is a neuronal

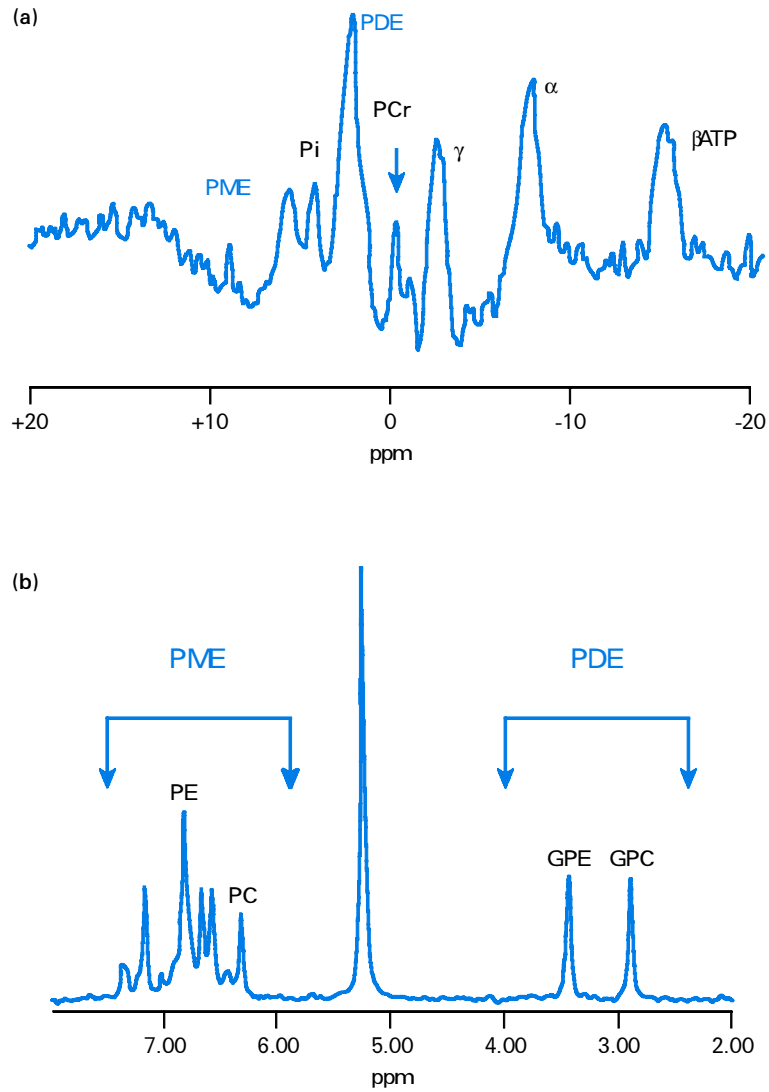

Fig 2. (a) A typical *in vivo* ^{31}P hepatic magnetic resonance (MR) spectrum from a healthy volunteer using two-dimensional chemical shift imaging technique (repetition time 1 s, pulse angle 45°). Resonances are assigned to phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE) and adenosine triphosphate (ATP).
(b) An *in vitro* ^{31}P hepatic MR spectrum from a non-cirrhotic liver. There is resolution of the PME and PDE resonances into their constituent components. Phosphocholine (PC) and phosphoethanolamine (PE), adenosine monophosphate (AMP) and glycolytic intermediates, such as glucose 6-phosphate, contribute to the PME peak. Phospholipid cell membrane degradation products (including glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE)) and endoplasmic reticulum are the main components of the PDE peak (PCr = phosphocreatine).

marker[3]. The 2.1–2.5 ppm region of the ^{1}H MR spectrum contains contributions from both glutamine and glutamate (Fig 3)[4].

Applications of magnetic resonance spectroscopy to liver transplantation and chronic liver disease

These studies have included:

- the functional assessment of hepatic failure in patients prior to orthotopic liver transplantation (OLT)
- the assessment of the viability of the isolated, stored donor liver before implantation into the recipient
- the early diagnosis of rejection post-OLT[5-16].

A prognostic indicator in chronic liver disease

The clinical spectrum and natural history of cirrhosis are varied, with some individuals having a rapidly progressive course and others relatively indolent disease. In mild disease, no active treatment is usually required, whereas OLT is the only form of treatment shown to prolong life in end-stage cirrhosis with hepatic failure. Accurate prognostic markers are necessary, because patients likely to remain reasonably well should be identified early so that they are not unnecessarily transplanted.

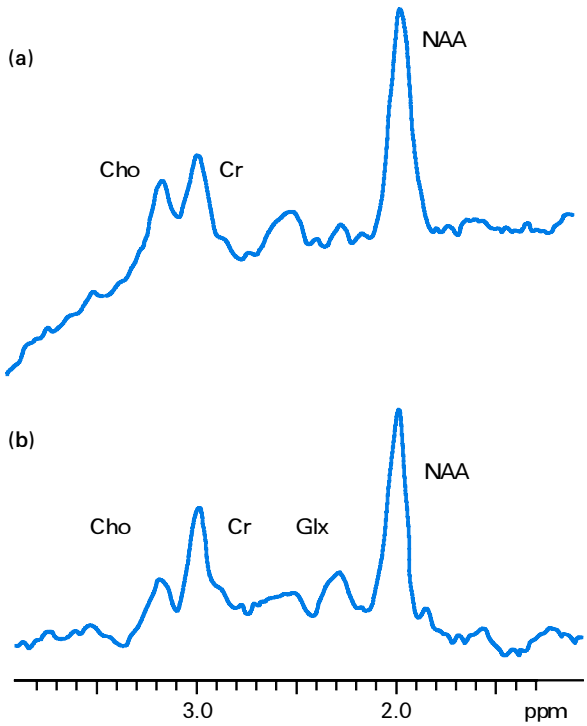

Fig 3. Two-dimensional chemical shift imaging (2-D CSI) spectra from the basal ganglia of (a) a normal volunteer and (b) a patient with overt chronic hepatic encephalopathy (repetition time: 1,500 ms, echo time 130 ms). There is an increase in the glutamine/glutamate (Glx)/creatine (Cr) ratio and a decrease in the choline (Cho)/Cr ratio in the patient's spectrum (NAA = N-acetylaspartate).

Conversely, those with rapidly progressive disease need to be detected before they become too ill to undergo OLT.

Our cross-sectional studies over the past eight years have established a close correlation between the abnormalities in phospholipid metabolism, measured by ^{31}P MRS, and the degree of hepatic decompensation. An increase in the PME/ATP and PME/PDE ratios, and a decrease in the PDE/ATP ratio are significantly related to the severity of liver dysfunction (Fig 4)[5–8]. Other research groups have described similar results[9]. The underlying biochemical mechanisms for these changes have been elucidated using *in vitro* MRS of liver biopsy samples obtained at liver transplantation[6,7]. The increase in PME is related to increased cell membrane precursors, such as PE and PC, while the decreased PDE is a reflection of reduced cell membrane degradation products, such as GPE and GPC, as these pathways are switched into 'regeneration mode' in an attempt to repair the failing liver[6]. In a pilot study of 22 patients with primary biliary cirrhosis, we have also shown a close correlation between these MRS-detectable abnormalities and the commonly used indices of disease outcome[10]. MRS may therefore provide insight into metabolic changes within the cirrhotic liver but, unlike many other dynamic liver function tests, the information obtained is not blood flow dependent. At present, a large proportion of this patient population undergoes regular imaging in many hospitals to screen for the development of hepatocellular carcinoma. In the future, MRS data might be obtained as an 'add-on' to a standard MRI examination.

Assessment of isolated donor liver viability

Organ transplantation is a rapidly expanding surgical field, and clinical pressure has led to a serious shortage of donor livers with the result that livers from suboptimal donors are sometimes used. There is no non-invasive methodology to assess the viability of these livers in the window of opportunity between organ harvesting and implantation into the recipient. A reliable assessment of stored organ metabolic status is important to prevent both organ wastage and the use of organs that fail to function post-operatively. The aim of our MRS studies has been to determine pre-transplant hepatic function in the preserved donor liver in order, ultimately, to allow better utilisation of the donor organ pool.

In the future, hepatic ^{31}P MRS may prove to be an ideal non-invasive tool for assessing isolated donor organ viability. ATP is pivotal to the maintenance of cellular homeostasis, and its presence can be measured effectively with MRS. Our initial research in the area of liver transplantation and the application of MRS has used both small[11] and large[12,13] animal models to evaluate the technique prior to its use on human donor livers. A pig liver model has been developed which exactly follows the human organ harvesting and storage protocol. This has established the rapid loss of ATP with harvesting and preservation of the pig liver in comparison to that seen with rodents, and demonstrated for the first time that ATP can be regenerated in a large animal liver by hypothermic reperfusion[13,14]. This model has enabled the relative merits of different preservation

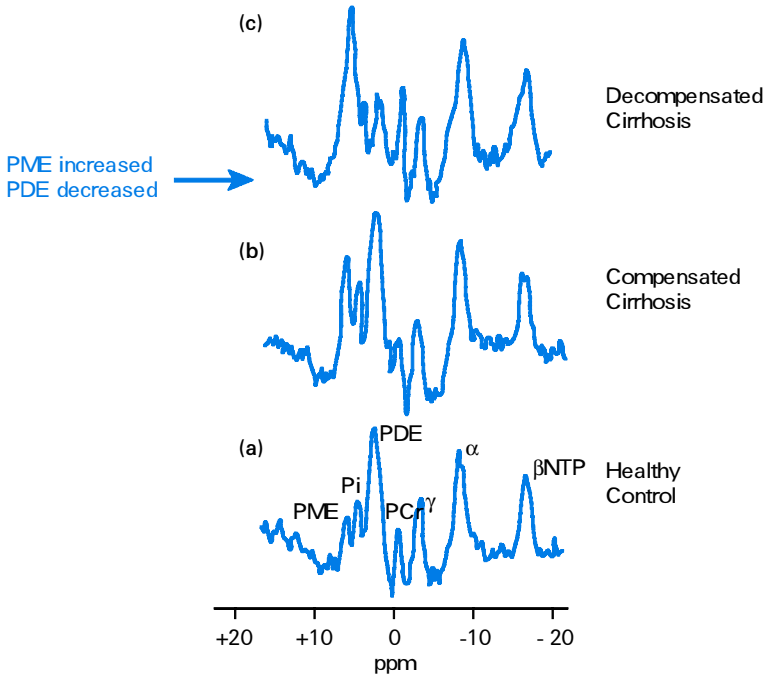

Fig 4. ***In vivo*** **hepatic ^{31}P magnetic resonance spectra acquired from (a) a healthy volunteer, b) a patient with functionally compensated cirrhosis, and c) a patient with functionally decompensated cirrhosis** (repetition time 5,000 ms).The phosphomonoester (PME) resonance increases and the phosphodiester (PDE) resonance reduces with worsening hepatic decompensation (NTP = nucleoside triphosphate; PCr = phosphocreatine; Pi = inorganic phosphate) (adapted from Ref 8).

solutions and methods of organ storage to be compared[15]. Purpose-built MRS probes have also been designed and built in such a fashion that they fit within human liver retrieval boxes. This eliminates any further sterility concerns with the liver once it has been retrieved, since all MR measurements require no physical contact with either the box or the liver. Human studies now need to be carried out to assess the utility of MRS in the detection of organ viability in a clinical transplant programme.

Assessment of allograft rejection after orthotopic liver transplantation

The major causes of morbidity, mortality and retransplantation are primary graft dysfunction (10–15%) and graft rejection, both acute (50–70%) and chronic (8–15%). The gold standard for diagnosis of acute rejection is histological examination of liver biopsy material, but there is no non-invasive marker that predicts rejection. This condition can usually be successfully treated by manipulation of anti-rejection chemotherapy, but early diagnosis is important to prevent the full-blown syndrome which may ultimately require retransplantation.

In vivo ^{31}P MRS animal studies have shown that an increase in the PME resonance correlates closely with acute rejection. Preliminary *in vivo* hepatic ^{31}P MRS studies of patients with chronic ductopenic rejection showed significantly elevated PME/ATP and PDE/ATP ratios, while patients with good graft function have normal spectra[16]. *In vivo/in vitro* ^{31}P MRS correlations were possible in three patients who were subsequently retransplanted. These results suggest that increased levels of cell membrane precursors are responsible for the abnormal PME/ATP ratio observed *in vivo*, while the rise in PDE/ATP ratio is probably due to an increased signal from either endoplasmic reticulum or bile in these severely cholestatic patients.

Cerebral magnetic resonance spectroscopy studies

The main area of interest in the brain in chronic liver disease is hepatic encephalopathy, which occurs in two distinct forms. First, in acute liver failure, the condition progresses rapidly, over a period of days – or even hours – with a high associated mortality if left untreated[17]. The predominant neuropathological picture is of cerebral oedema, due to increased permeability of the blood-brain barrier[18]. Death may result from cerebral coning, with herniation of the brain through the basilar foramen as a result of increased intracranial pressure from the development of cerebral oedema[18].

Secondly, the neuropsychiatric abnormalities affecting patients with chronic liver disease are termed portal-systemic encephalopathy or chronic hepatic encephalopathy (CHE). This condition results from either the development of hepatocellular failure or a portal-systemic collateral circulation in patients with chronic liver disease. This may arise as a complication of portal hypertension or from the surgical placement of a portal-systemic shunt, usually for the management of uncontrolled variceal bleeding[19]. Such shunts may be performed by standard surgical techniques or by using transjugular intrahepatic portal-systemic stent shunts (TIPSS) placed under radiological guidance. In most patients, encephalopathy results from a combination of these factors.

CHE is usually minimal in the majority of patients, affecting reaction times in activities of daily living such as driving and

Key Points

- ***In vivo* magnetic resonance spectroscopy (MRS) provides a non-invasive means of studying body biochemistry**
- **MRS can be performed as an 'add-on' sequence at the end of most clinical MR imaging examinations**
- ***In vivo* ^{31}P MRS provides objective functional information on the failing liver, the viability of donor organs prior to transplantation, and the integrity of the graft following liver transplantation**
- ***In vivo* MRS delineates cerebral metabolite abnormalities in hepatic encephalopathy and in patients with chronic hepatitis C infection**
- **The technique may be used in the future as a non-invasive marker of gene delivery**

operating machinery[20]. It is usually detected in slowing of the EEG frequency or as impairment of psychometric performance[21–23]. However, the syndrome may become clinically overt in about 30% of patients with chronic liver disease, with alterations in behaviour, mood and personality, and disturbances in consciousness[21]. The condition is usually characterised by remissions and relapses, although in some patients the neuropsychiatric impairment may follow a more persistent course.

The pathogenic mechanisms underlying CHE are only just beginning to be unravelled. Direct investigation in man has become possible recently with imaging techniques such as MRS and positron emission tomography. Circulating toxins and changes in the functional state of cerebral neurotransmitter systems have been causally implicated in the genesis of the condition, with alterations in cerebral glutamine, glutamate and ammonia metabolism of importance. *In vivo* ^{31}P MRS can be used to provide non-invasive biochemical information on high energy phosphates, membrane turnover and glycolytic intermediates in the brain[24–26]. Several MRS studies suggest disordered brain energy metabolism, with changes in PCr and Pi[27–29] and decreased measurable ATP levels even in patients with minimal encephalopathy[26]. Other abnormalities in the cerebral spectra suggest that glucose utilisation is decreased and phospholipid metabolism altered with reductions in both the PME and PDE resonances, possibly reflecting changes in the blood-brain barrier in these individuals[24–26]. These spectral changes have been correlated with the underlying severity of neuropsychiatric impairment[24]. ^{1}H MRS studies on the brains of CHE patients show an increased glutamate resonance, reflecting ammonia detoxification in astrocytes[25,26,30–35], and reduced Cho and myoinositol resonances[25,26,30–35]. The reduction in myoinositol, a cerebral osmolyte, reflects the attempts of the brain to control for cell swelling[34]. All these ^{1}H MRS results correlate closely with the severity of encephalopathy (Fig 3)[30]. Spectral changes improve following treatment, so MRS may also prove to be a useful objective measure in disease monitoring and in gauging response to new treatment regimens.

Hepatitis C

Patients with chronic hepatitis C virus (HCV) infection frequently complain of symptoms similar to those of chronic fatigue syndrome, often in the absence of clinically significant liver disease. Objectively, they score lower on quality of life scores than patients with chronic hepatitis B virus (HBV) infection[36]. We now use ^{1}H MRS to investigate whether there are cerebral metabolite abnormalities in HCV infected individuals with minimal or clinically insignificant liver disease[37]. Thus far, we have found that the Cho/Cr ratio is significantly elevated in the basal ganglia and white matter of patients with HCV, compared to both a HBV group with equivalent liver disease and healthy volunteers. There have been no differences between the HCV patients with or without a history of intravenous drug usage.

These results closely mirror those seen in cerebral HIV infection in their nature and anatomical distribution[38,39], raising the possibility that HCV may also infect the central nervous system. It is important to note that the changes are qualitatively different from hepatic encephalopathy, particularly with respect to the Cho resonance. Furthermore, this is unlikely to be a confounding factor, since all patients had histologically-proven mild or minimal liver disease with no evidence of significant inflammation or fibrosis, or of cirrhosis on biopsy. It remains to be seen what these spectral abnormalities mean, but direct studies on the brains of patients dying with hepatitis C are needed to determine whether the virus infects, rather than affects, the brain.

Future developments

Gene therapy for the liver

Recent advances in MRI and MRS afford the possibility of detecting and assessing transfer, expression and subsequent therapeutic changes of effector or marker transgenes non-invasively. In the field of MRI, 'smart' MR contrast agents are being developed, so-called because they change their conformational structure and, in so doing, induce MR detectable changes in a given tissue. These agents become 'switched on' in response to physiological changes brought about by the enzymatic action of a given gene product (enzymes), and are being developed for use in intact cells, isolated organs and animal models. Ultimately, these agents hold the promise of bridging the gap between the laboratory and the patient with non-invasive detection of transgene expression *in vivo* in man[40,41].

MRS is being developed as a non-invasive method to assess transgene expression indirectly by means of MR visible intracellular markers. These markers take the form of intracellular endo/exogenous metabolites associated with exogenous enzyme expression and function[42]. This technique will be applicable to a variety of different situations, from cell suspensions through to clinical imaging of the whole body. For example, PCr is not expressed in healthy hepatocytes, but the introduction of the gene for Cr kinase might act as an MR visible marker of gene

delivery, since a PCr resonance would appear in the liver where one was not present before[42].

Conclusions

Hepatic and cerebral MRS allows a serial non-invasive insight into metabolism *in vivo*. This technique holds promise both for disease diagnosis and for monitoring the biochemical response to treatment. It also has scientific value as a source of information on the functional basis of disease. Although MRS cannot yet be regarded as a clinical tool in routine practice, it has exciting prospects, such as in the burgeoning field of gene therapy directed to the liver.

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