Nuclear magnetic resonance in clinical pharmacology and measurement of therapeutic response

W. H. AELLIG

Clinical Research, Sandoz Ltd, Basel, Switzerland

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Introduction

It is essential for the clinical pharmacologist to assess new methods in medicine and biology for their potential applications. The main interest in this context lies in non-invasive, accurate and easily reproducible techniques for measuring drug effects and their time-course. The purpose of this article is to review the potential of nuclear magnetic resonance imaging and spectroscopy for clinical pharmacological studies. Technical details will be simplified and restricted to those essential for a proper understanding of the basic principles of this new method. Any reader interested in more detail is referred to the list of references. It must be mentioned at the outset that the equipment needed for the clinical use of nuclear magnetic resonance (NMR) is expensive, so it is unlikely that any clinical pharmacology department will be able to afford it for its exclusive use. With the increasing number of installations in hospitals, however, they will become accessible also to the clinical pharmacologist. Not many investigations have yet been carried out using NMR to answer clinical pharmacological questions. The evidence available from other studies, mainly in the diagnostic area, will however help to introduce the clinical pharmacologist to this new technique and illustrate its potential advantages in clinical pharmacology.

Physical basis of NMR

Nuclear magnetic resonance is an established method in chemical analysis which is now also gaining interest and importance in the fields of diagnosis and medical research. A series of atomic nuclei, such as those of hydrogen

(protons), ^{31}P , ^{13}C , ^{23}Na , ^{19}F , etc., possess an inherent spin. The rotating movement of an electrically loaded particle creates a magnetic field along the spin axis. Under normal conditions this magnetic field is not detectable because the spin axes of the many nuclei present in a given sample (e.g. the human body) are randomly oriented in all spatial directions so that the net magnetisation, i.e. the sum of all magnetic fields, is zero. In a strong magnetic field, however, the spinning nuclei align in a direction parallel or antiparallel to the field axis, with a small preponderance in the parallel state, thus producing a net magnetization parallel to the applied field. In this strong magnetic field the spinning nuclei behave like spinning-tops in the gravitational field of the earth. They experience a torque which makes them move around their spin axes so that their axis of rotation describes a cone. This movement is called precession and it takes place at a given frequency, called Larmor frequency, which depends on the atomic nucleus (for magnetic resonance imaging usually protons) and the strength of the magnetic field. With the magnetic fields used in clinical magnetic resonance imaging and spectroscopy, the Larmor frequencies are within the range of standard radio frequencies (RF).

We can now apply to our sample a short pulse of electromagnetic waves of the appropriate Larmor frequency. If this is done in a direction perpendicular to the main field, the spinning nuclei experience a torque which makes them precess at a given angle to the main field and synchronized at the Larmor frequency. This now produces a magnetization perpendicular to the field axis oscillating at the Larmor frequency. If the radiofrequency is switched off, the syn-

chronized precessing spins, representing small moving magnets, induce a voltage in the radio receiver coil with a frequency identical to their Larmor frequency.

It is this RF emission which can be used to gain information on the sample placed in the magnet. If the strength of the magnetic field gradually changes along a given axis of the sample (this can be done with the aid of gradient coils), the resonance frequency of a given nucleus depends on its position on this axis. By analysing the frequencies of the emitted RF waves, it is therefore possible to determine from which position on this axis a particular signal originates. Magnetic field gradients can be applied along the three dimensions of space, so that it is possible to locate signals from each volume element (voxel) of the given sample. A combined analysis of all of these signals therefore allows the construction of images of the object placed in the magnet. This is the basis for magnetic resonance imaging (MRI) (e.g. Kramer, 1984; Seelig, 1986). The intensity (or brightness) of the different points of a picture (the picture elements or pixels) depends on the one hand on the relative density of protons (the nucleus most commonly used for imaging) in each pixel, on physical parameters

determined by the chemical composition of the object (see below) and on the particular sequence of RF pulses used.

Magnetic resonance imaging (MRI)

For magnetic resonance imaging the human body is placed into the bore of a large magnet with a strong field (Figure 1). Most of the magnets used at present have fields ranging from about 0.15 to 2 tesla (1 tesla = 10,000 gauss); for comparison, the magnetic field of the earth amounts to 0.3 to 0.7 gauss depending on the geographical position. RF pulses are applied by RF coils placed inside the magnet. The re-emitted RF waves are received and processed by Fourier methods leading to tomographic pictures of the different regions of the human body analogous to those obtained with an X-ray computer tomograph (CT). There is, however, a fundamental difference between the two methods. The signal intensity of the picture elements on a CT scan depends on the X-ray absorption of the different organs, whereas on an MR image it is due to the density of protons and the chemical composition of the organs. This difference is responsible for

Figure 1 Photograph of the clinical magnetic resonance device at the MR center at Basel University (Photograph Siemens).

the fact that CT and MR pictures emphasize different aspects of the tissues and the organs investigated, thus permitting additional information to be gained in several diagnostic areas. In contrast to CT, MRI allows images to be obtained in all possible orientations of the body. The standard orientations available on all instruments are frontal, axial and sagittal, but also oblique and double-oblique scans (tilted along one or two axes) are possible.

In addition, and this is of special importance if many pictures of the same patient are required, MRI uses non-ionising radio waves, whereas CT requires ionising radiation. With the magnetic fields and RF pulse sequences used at present, MRI is considered to be without harmful effects to the subject investigated (Consensus Conference, 1988).

A standard MR image consists of 128×128 or 256×256 pixels and typically requires 128 or 256 measurements of about 1 s duration. The acquisition of such an image therefore takes a certain time. This means that when a moving structure, such as the heart, is imaged, if no precautions were taken, each measurement

would be made at a different time of the cardiac cycle. To avoid this, ECG triggering is used, which starts the acquisition of each subsequent measurement at exactly the same time point during a cardiac cycle. This means that the time interval between the acquisition of two measurements is at least one heart beat. The time required to obtain a full picture of the heart at a given time point during the cardiac cycle is therefore at least 128 or 256 heart beats. This disadvantage of the relatively long time required to obtain one scan is partly mitigated by the fact that the waiting time between heart beats can be used to initiate the acquisition of other images of the heart at different time points during the cardiac cycle, usually on different levels of the organ.

Apart from proton density, the contrast of the MR image depends also on the so-called relaxation times T_1 and T_2 , two physical parameters reflecting the chemical composition of the object investigated. T_1 represents the time it takes for the excited nuclei to return to their equilibrium state after the RF pulse has been turned off (typically 300 ms to 1 s). T_2 represents the time it takes for the excited spinning nuclei to lose

Figure 2 Images can be obtained in all spatial orientations. The figure shows a sagittal (upper left) and a frontal (upper right) view of the human thorax. ECG triggering allows image acquisitions at different times during the cardiac cycle. The lower part of the figure shows a diastolic (left) and a systolic (right) axial image of the heart.

phase coherence of their precession (usually a few hundred ms), mainly due to fields induced by other nuclei. For a given magnetic field, T_1 and T_2 depend on the chemical composition of the sample studied (T_1 of water, for example, is much longer than that of very viscous liquids) and allow therefore some differentiation of tissue types and pathological states of tissues.

Clinical applications of MRI

There are several fields of clinical investigation where the practical applications of MRI are already established mainly for diagnostic purposes (see Pohost & Canby 1987; Stark & Bradley, 1988; Steiner, 1986a). One of these is the study of the central nervous system. Bone, which has a high X-ray density, gives only a very low signal in MRI. There is, therefore, no interference with an investigation of organs surrounded by bones. In this important area, diagnostic MRI has facilitated not only the diagnosis of many tumours but also the quantitative investigation of cerebral bleeding, oedema and ischaemic infarction as well as the diagnosis of multiple sclerosis (Bradley *et al.*, 1984; Bryan *et al.*, 1983; Drayer, 1988; Langner *et al.*, 1986; Ormerod *et al.*, 1984). In neonates MRI allows the observation of structural development and myelination of the human brain (Martin *et al.*, 1988).

In the area of cardiovascular research, pictures of the heart in all possible planes can be obtained (Figure 2) which can be used for an exact determination of the size of the different cavities and myocardial wall thickness and mass (Just *et al.*, 1987; Katz *et al.*, 1988; Sechtem *et al.*, 1987a; Steiner *et al.*, 1983, 1987b; Underwood *et al.*, 1988). Also the determination of liver volumes is possible with such a technique (McNeal *et al.*, 1988).

Attempts have also been made to use changes in T_1 and T_2 times for clinical diagnostic purposes (Bottomley *et al.*, 1987a), including the differentiation between malignant metastatic and non-neoplastic lesions in the bone marrow (Sugimura *et al.*, 1987), the diagnosis of human cardiac allograft rejection (Wisenberg *et al.*, 1987 see below), the investigation of the time course of myocardial infarction (Been *et al.*, 1988) and the characterization of subclinical myocardial necrosis (Ahmad *et al.*, 1988).

Although MRI does not require contrast agents for most of its applications, there have been investigations with several agents which influence relaxation times of nuclear spins, and one (gadolinium DTPA) is available for clinical use at present. This paramagnetic agent,

which reduces T_1 and T_2 values in the tissues in which it is distributed, has been used to alter the contrast behaviour of certain tissues and has been found useful e.g. in detecting active lesions in multiple sclerosis (Grossman *et al.*, 1988) and cerebral metastases (Healy *et al.*, 1987; Russell *et al.*, 1987).

Functional images

A major potential of MRI is that it can be used to obtain not only static but also dynamic images reflecting different phases of an event, such as the cardiac cycle and blood flow. The application of rapid imaging sequences, such as FLASH (Frahm *et al.*, 1986) helps to reduce measuring time considerably. This enables the construction of pictures at many time points (usually about 12–16) during the cardiac cycle. Such images of the heart obtained on the one hand at many different times throughout the cardiac cycle (Figure 3) and on the other hand in parallel slices (usually 8–10) from the valves to the apex make possible the determination of a series of functional parameters, such as stroke volume and ejection fraction (Møgelvang *et al.*, 1986; Rienmüller *et al.*, 1987; Sechtem *et al.*, 1987a, b), but also left ventricular filling and ejection rates. If taken in a standardized manner, these non-invasive pictures and the functional analyses derived from them are reproducible even over longer periods of time.

Other functional investigations using MRI include measurement of blood velocity and flow (see below) and renal function (Kikinis *et al.*, 1987).

Potential of MRI in Clinical Pharmacology

A special advantage of MRI is the fact that reproducible images can be obtained repeatedly over prolonged periods of time without either the problem of irradiation, as with X-ray methods, or the problem of exactly positioning the probe as with echo methods. This provides the opportunity to follow the time course of the effects of therapeutic interventions. Only few such studies have yet been carried out, since the method is still relatively new, but many different potential applications can be imagined. Some typical examples will be discussed, which may stimulate further ideas for projects in other therapeutic or experimental areas.

A great potential of NMR for clinical pharmacology lies in the cardiovascular field. MRI is probably the most accurate method for the non-invasive assessment of ventricular volumes (Editorial, 1988). It can also be used to deter-

Figure 3 With fast sequences images can be obtained at many time points throughout the cardiac cycle. The Figure shows nine images through a midventricular plane perpendicular to the long axis of the heart obtained at 50 ms intervals.

mine myocardial mass. Although MRI investigations are more time consuming and more expensive than e.g. echocardiography (which is of course well suited for acute diagnostic studies), they have the great advantage of easy reproducibility and relatively low operator dependence. Not only functional parameters (such as stroke volume and ejection function) can be determined, but also e.g. the time course of left ventricular volume during the cardiac cycle and therefore e.g. left ventricular filling and ejection rate. Determination of these parameters before and during chronic oral therapy of patients with essential hypertension may help to evaluate differential effects of such therapy on various pathophysiological parameters. Similar studies are possible in patients with congestive heart failure.

Another interesting clinical pharmacological application is the investigation of the regression of left ventricular hypertrophy during treatment of patients with essential hypertension (Eichstaedt *et al.*, 1987). The new fast multislice techniques will allow the determination of the time course of the regression of left ventricular hypertrophy

and at the same time parameters of cardiac function such as ejection fraction and stroke volume.

Rubinstein *et al.*, 1987, demonstrated the potential of MRI in the evaluation of the patency of aortocoronary bypass grafts. Ahmed *et al.* (1988), suggested the measurement of T_2 values in human myocardium as a potential indicator for severely ischaemic tissue.

In patients with heart transplants, followed over 4 years, Rienmüller *et al.* (1989) found, that the frequency of histological rejection episodes correlated with a reduction in left ventricular mass. From studies in the rat, the determination of changes in T_1 and T_2 values has been proposed as a potential indicator for cardiac allograft rejection (Sasaguri *et al.*, 1985). Preliminary studies in man (Wisenberg *et al.*, 1986, 1987) look promising; the data indicate a prolongation of T_1 and T_2 values, but unfortunately the results are discriminatory only 3–4 weeks after surgery because earlier on T_1 and T_2 are prolonged also in non-rejected grafts. If further studies confirm these data NMR investigations (see also MRS-studies in cardiac allografts below) may well

reduce the need for cardiac biopsies for the monitoring of immunosuppressive therapy in patients with cardiac transplantations.

Studies carried out in animals (Rudin *et al.*, 1988) and man (Lundberg *et al.*, 1986) show that NMR is also well suited to observe the regression of hypophyseal prolactinomas during appropriate therapy. The influence of a calcium antagonist (isradipine) on the extent of the brain infarct occurring after occlusion of the middle cerebral artery was studied with MRI in rats and a reduction observed in the treated animals (Sauter & Rudin, 1986). Studies on the time course of cerebral infarction and the potential influence of therapy may therefore be possible also in man.

Janus *et al.* (1988) used MRI for the examination of follicular and endometrial development during the menstrual cycle and suggested its application for the monitoring of therapeutic interventions in infertile women. Pettigrew *et al.* (1986) showed that the use of the paramagnetic contrast agent Gd-DTPA may be useful to gain at the same time morphological and physiological information on liver and kidney. Such investigations may be useful to study the effects of therapeutic interventions.

The different behaviour of moving spins, which may produce artefacts in standard MR images owing to moving blood, can also be exploited for special applications, such as angiography (Dumoulin & Hart, 1986) including venography (Edelman *et al.*, 1989) and the measurement of arterial blood flow (Edelman *et al.*, 1986; Müller *et al.*, 1986) as well as e.g. the determination of regurgitant volumes in patients with aortic valvular lesions (Sechtem *et al.*, 1988) or the blood flow pattern in the human aorta (Klipstein *et al.*, 1987). Methods are also in development for the study of the evolution of arteriosclerotic lesions (Herfkens *et al.*, 1983; Mohiaddin *et al.*, 1989a, b) during appropriate therapy.

NMR spectroscopy

As explained above, each magnetic nucleus is characterized by its own resonance (or Larmor) frequency. Larmor frequencies of the different atomic nuclei differ by several MHz so that separate experiments are required when investigating more than one type of nucleus. Even for different members one single class of magnetic nuclei such as protons, however, small differences in resonance frequencies of the order of a few Hz can be observed. These are caused by the modulation of the magnetic environment due to

Figure 4 ^{31}P magnetic resonance spectrum of the human calf muscle. Pi = inorganic phosphate, PCr = phosphocreatine. α , β , γ = the three peaks corresponding to the three P-atoms in ATP (with kind permission from Seelig, 1986).

the chemical bonding pattern. This fact is called chemical shift and has long been used for analytical NMR spectroscopy in chemistry. It can now also be used for certain biochemical investigations in animals and man *in vivo*. An example is given in Figure 4, which shows that even the different P-atoms of ATP can be distinguished from one another (Seelig, 1986).

The stable isotopes most frequently used for *in vivo* MR spectroscopy (MRS) are ^{31}P , ^{13}C , ^1H , and ^{19}F (^{31}P , ^1H and ^{19}F have a natural abundance of 100%, ^{13}C of only 1.1%). Resonance spectra from ^{31}P of human muscle allow the *in vivo* determination of the relative amounts of phosphocreatine, ATP, and inorganic phosphate present in a given muscle sample but, by measuring the distance between phosphocreatine and inorganic phosphate peaks the determination of the intracellular pH value is also possible. ^{13}C resonance spectra allow e.g. measurements of glucose uptake and metabolism and the determination of the composition of fatty acids in human tissue.

Applications of NMR spectroscopy in Clinical Pharmacology

A comprehensive review of the different chemical substances which have until now been determined with *in vivo* MRS and the potential of human MRS for clinical diagnosis and clinical research has recently been published by Bottomley *et al.* (1987a, 1989). From the evidence available, many practical applications can be imagined in which the determination of changes in the chemical composition of e.g. muscle or brain regions could be useful in clinical pharmacology. The methodology here is, however, not

yet so far advanced as in MRI, but some promising initial results are available. Using ^{31}P MRS, one such investigation confirmed the reduction of phosphocreatine and the resultant rise of inorganic phosphate in the human calf muscles during physical exercise. The time course of these changes was found to be similar in healthy subjects and patients with arteriosclerotic lesions. After the end of exercise, however, baseline values were reached much more rapidly in healthy subjects than in arteriosclerotic patients (Keller *et al.* 1985). This method will therefore allow the study of the effects of potential therapies in this indication. Forearm skeletal muscle metabolism of high-energy phosphates was also investigated in patients with congestive heart failure (Massie *et al.*, 1987; Wiener *et al.*, 1986). There are indications that at least in a subset of patients this is impaired. The changes observed did not correlate with the reductions in forearm blood flow. It might therefore be interesting to investigate the effects of different forms of therapy on these parameters and to correlate them with their effects on exercise tolerance. The non-invasive study of high-energy phosphate metabolism of human heart *in vivo* has also been suggested (Bottomley, 1985; Conway *et al.*, 1989; Müller *et al.*, 1988). Sophisticated studies are already possible in isolated perfused animal hearts (Auffermann *et al.*, 1988). In man this is at present technically still difficult because only volumes near the body surface can be studied. Recent investigations by Bottomley *et al.* (1987b), however, suggest that ^{31}P -MRS may also be useful for the direct assessment of cellular energy reserve in clinical myocardial infarction and to evaluate the metabolic response to interventions.

Studies in the dog (Chacko *et al.*, 1988) suggest that some ^{31}P MRS parameters (the ratio of phosphocreatine to inorganic phosphate concentration and the ratio of phosphocreatine to β -ATP concentration) may be good predictors of graft rejection after cardiac transplantation. Some promising initial results in man have also been reported with a good correlation with the results of endocardial biopsies (Herfkens *et al.*, 1988).

In vivo investigations of human tumours of different origin suggest that ^{31}P MRS may be helpful for their characterization but probably also for the evaluation of the effects of therapeutic interventions (Cox *et al.*, 1988; Oberhänsli *et al.*, 1986).

Metabolic studies in the brain are also possible, either with ^{31}P (high-energy phosphates (Hamilton *et al.*, 1986)) or with ^1H (e.g. cholines, N-acetyl aspartate, glutamate and lactate

(Frahm *et al.*, 1988; Hanstock *et al.*, 1988)). The effects of acute stroke on cerebral metabolism have also been studied (Bruhn *et al.*, 1989); the method may also be useful to study the effects of therapeutic interventions.

Glucose and glycogen metabolism can be investigated by means of ^{13}C -MRS. In such a study carried out in rats, Pahl-Wostl (1987, personal communication) found that the administration of a somatostatin analogue (octreotide) markedly reduced the amount of ^{13}C -glycogen formed in the liver after i.v. infusion of ^{13}C -glucose and slowed down ^{13}C -glycogen breakdown after the end of the infusion. Hepatic and muscle glycogen can be labelled by the ingestion or injection of ^{13}C -glucose. In healthy subjects this leads to an immediate increase in ^{13}C -glycogen, whereas in diabetics at first ^{13}C -glucose appears in the muscle and only later ^{13}C -glycogen; this shows delayed glycogen synthesis (Jue *et al.*, 1988). ^{13}C -NMR studies in healthy subjects and in patients with non-insulin dependent diabetes mellitus (NIDDM) showed, that muscle glycogen synthesis is the major pathway of post prandial glucose disposal and that defects in muscle glycogen synthesis play a major role in the impaired glucose disposal in NIDDM patients (Shulman *et al.*, 1989). ^{13}C -NMR of human muscle cells *in vivo* has therefore the potential for investigating not only pathophysiological changes in glucose and glycogen metabolism, but also the effects of therapy in diabetic patients.

Oberhänsli *et al.* (1987) studied the effects of fructose on liver metabolism in patients with hereditary fructose intolerance. The quantification of osteoporotic changes by NMR spectroscopy has also been suggested (Brown *et al.*, 1987a, b).

It would be fascinating to carry out direct measurements of drug concentrations in the human body by using NMR spectroscopy. Unfortunately this is not possible for most substances because the present sensitivity of the method only enables measurement of concentrations of about 1mm or higher, whereas the therapeutic plasma concentrations of most drugs are considerably lower. Some promising results have, however, been reported regarding the kinetics and metabolism of 5-fluorouracil studied by means of ^{19}F spectroscopy (Semmler *et al.*, 1988).

From the interesting data available at this early stage, we can expect that in the future NMR spectroscopy will permit us to determine many more biochemical changes in living man not only during different diseases but also during their acute and long-term therapy.

Safety and inconvenience

MRI and MRS are generally considered safe, provided the limitations specified e.g. by the FDA or the British NRPB are adhered to. No adverse effects of the magnetic fields as such have been observed at the levels used on currently available clinical devices (Consensus Conference, 1988). Probably the most important inherent danger is the strong attraction of ferro-magnetic objects by the magnet, which may lead to projectile effects. All such objects have therefore to be left behind before entering the magnet room. Care has also to be exercised in patients with metallic implants, especially ferromagnetic surgical clips. For other implants such as osteo-synthetic plates etc. heating effects due to the magnetic RF fields were feared, but in recent experimental studies no temperature rise was found (Buchli *et al.*, 1988).

One problem often observed in MR studies is the anxiety of patients who have to lie in the rather narrow tube of the magnet for relatively long periods of time and to hear the relatively loud noise created by switching the magnetic gradients. In some patients, symptoms of claustrophobia may even preclude MR investigations, but a recent analysis suggested that anxiety was most frequent if no appropriate information of the procedure had been given to the patient (Quirk *et al.*, 1989). Temporary and mild hearing loss was also documented in some patients imaged but could be prevented by their wearing earplugs (Brummet *et al.*, 1988). Patients with cardiac pacemakers have to be excluded

from MR studies, as the procedure may interfere with the safe working of their device (Consensus Conference, 1988). Another problem is anaesthesia, because currently used anaesthetic equipment contains many ferromagnetic parts and some of the monitoring devices may not function properly in strong magnetic fields. Modifications have, however, been suggested that may provide safe anaesthesia and patient monitoring also in patients undergoing MR investigations (Boesch *et al.*, 1988; Karlik *et al.*, 1988).

Conclusions

Nuclear magnetic resonance imaging and nuclear magnetic resonance spectroscopy are relatively new and very promising methods in medical research. They permit the acquisition of detailed and accurate images of many organs in the human body with a high information content without the use of ionising radiation. In addition, functional non-invasive studies and biochemical investigations, which have hitherto not been possible, can be carried out in man *in vivo*. These new methods are still in rapid development and expected to open up a large variety of new opportunities for non-invasive investigations. These will provide us with new and accurate tools not only for the diagnosis of many diseases, but also for more accurate observations of the time course of diseases and the effects of therapeutic interventions on morphological, functional and biochemical parameters.

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