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Invited Review

Tissutal imaging by nuclear magnetic resonance

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Summary. The present work reviews the main applications of nuclear magnetic resonance (NMR)-technology and, in particular, of magnetic resonance imaging (MRI) to tissutal analysis. To date, MRI represents a precise and reliable tool to investigate morphology and functional modification of tissues *in vivo*, providing information consistent with histology. MRI has numerous advantages over conventional techniques: it is harmless to tissues; volume measurements *in vivo* could be useful for morphometric studies; the same tissue can be examined several times (e.g. at different ages); several organs can be examined at the same time; serial sections of relevant structures can be obtained in all planes, thereby allowing detailed reconstruction of the three-dimensional configuration of organs; motion within a tissue can be detected; and subsequent histological and ultrastructural studies of the tissue are possible. The main drawback (besides the cost of the basic instrumentation) is that resolution is relatively low in comparison with light microscopy. Finally, the analysis of the results is difficult, needing interdisciplinary competence, and MRI methods of tissutal analysis are not yet well standardized. Therefore, in our opinion, MRI is an interesting tool, complementary to other histological techniques, and it cannot be ignored by microscopists. However, *in vivo* MRI data must be evaluated with caution and histological controls are always required.

Key words: Magnetic resonance imaging, MR-microimaging, Magnetic resonance microscopy, MR-microscopy, Histology

Introduction

In recent years, the importance of nuclear magnetic resonance (NMR)-technology in biomedical research has progressively increased. In NMR, the signal emitted during relaxation of atomic nuclei placed in a magnetic field after application of an appropriate radio-frequency pulse, provides detailed data on the chemical composition of living tissues, which can in turn be utilized to obtain computer-generated images (Lauterbur, 1973). The basic techniques were developed during the 1950s as a spectroscopic tool for biochemical analysis and, in the 1970s, instruments for magnetic resonance imaging (MRI) were produced. In recent years, MRI has become an increasingly appreciated diagnostic tool in medicine.

Recently, the technological development of these instruments has led to NMR-microscopes with a spatial resolution ranging between 10 to 200 microns, depending on the characteristics of both the sample and the instrument (Aguayo et al., 1986; Eccles and Callaghan, 1986; Johnson et al., 1986; Rudin, 1987; Harrison et al., 1988; Kuhn, 1990; Mallard, 1991; Callaghan, 1991). NMR microscopy with 4-micron resolution was also performed (Cho et al., 1988). In histology and histopathology, the high cost and complexity of the MRI-equipment have prevented widespread use of this new technology that is presently applied in only a few big centres. However, several studies have demonstrated the great potential of these instruments when used in correlation with more traditional methods of light and electron microscopy (Johnson et al., 1993). Our own group has utilized MRI since 1986 to approach problems of tissutal analysis that are difficult to solve by conventional microscopical methods alone. The present work reviews the main applications of MRI to tissutal analysis and cell biology (Fig. 1) with the aim to provide scientists involved in histology and histopathology information about the use of NMR-technology in clinical diagnosis and experimental paradigms, and to describe the state of the art and its future directions.

Functional morphology

The main advantage of MRI with respect to

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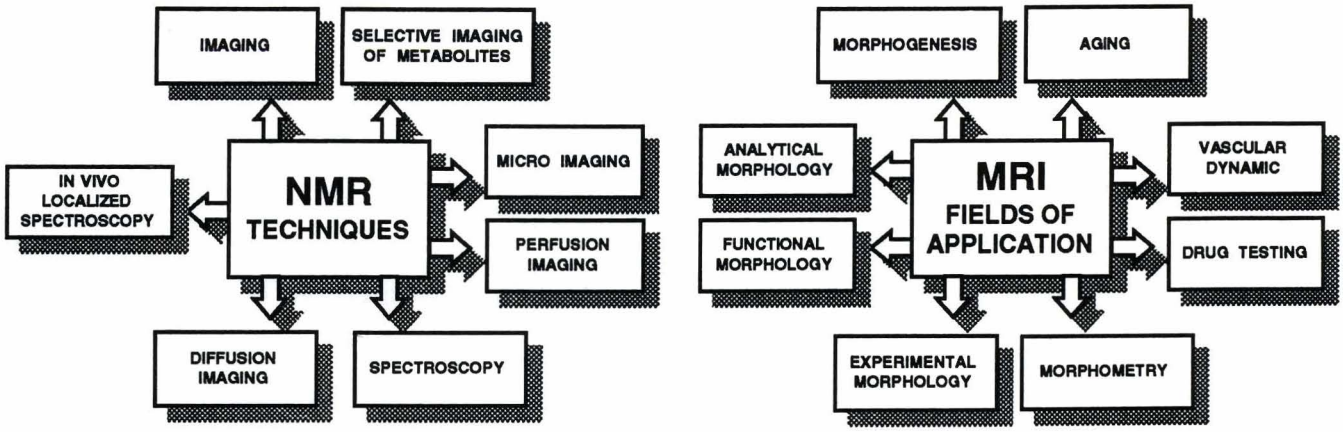

Fig. 1. Schematic summary of some NMR-techniques and MRI-fields of application.

conventional histological techniques is the possibility to study living tissue. In light microscopy, living tissue can usually be observed only *in vitro* after separation of the cellular elements. By MRI, it is possible to study, at a microscopic scale, the three-dimensional morphology of living animals (Bone et al., 1986; Johnson et al., 1987). Organs removed from the body and maintained under adequate perfusion condition can also be studied in morphological and biochemical aspects. MRI is harmless and does not require fixation or embedding procedures. For these characteristics, MRI is well suited for studies of tissue dynamics. With respect to other microscopic techniques, MRI permits repeated examination of the same tissue at different times, thus making it useful in the follow up of dynamic events. For example, it is possible to obtain a real time measurement of flow (Guilfoyle et al., 1991), movement of vascular wall (Behling et al., 1989) or a time course of secretions (Sbarbati et al., 1994, 1995). Functional activation of adipose tissue during hormone administration can be studied (Sbarbati et al., 1991a).

Morphological events can be correlated to adipocyte biochemistry using paradigm including NMR spectroscopy and ultrastructure (Zancanaro et al., 1994). Evaluating the time course of events by conventional morphological techniques is usually difficult requiring statistical evaluations between different groups of specimens. MRI adds a high temporal resolution to its high spatial resolution providing an excellent representation of dynamic processes at a submillimetric scale (Sbarbati et al., 1995). Several studies have demonstrated that MRI *in vivo* evaluations are consistent with subsequent histological evaluations. Therefore, MRI provides the possibility of a morphological approach to events that, in the past, were only studied by physiological or biochemical techniques, thus opening a new era in the field of functional morphology. In particular, many important studies have demonstrated the morphological aspect of the functional activation of the cerebral cortex by different stimuli.

These studies have mainly been performed by MRI techniques that can be used to obtain images in a fraction of a second rather than in minutes, such as the echo-planar imaging (Stehling et al., 1991). There is evidence that a local elevation in brain venous-blood oxygenation accompanies an increase in neuronal activity. The amount of oxygen carried by hemoglobin affects the magnetic properties of hemoglobin and MRI can detect these small magnetic fluctuations (Ogawa et al., 1990). Therefore, MRI machines can detect functionally-induced changes in blood oxygenation. To date, MRI studies of cerebral activation on the basis of oxygenation difference maps include the visual system, motorsensory system, and areas associated with language processing (Frahm et al., 1992; Kwong et al., 1992; Ogawa et al., 1992; Kim et al., 1993; Le Bihan et al., 1993; Turner et al., 1993). MRI studies of the functional activation of other organs are still scarce and this will surely be an important field of research in the future.

Age-related modification

MRI has found a large application in studies in morphogenesis, development, and aging. This is obviously due to the possibility to study the same structure at different ages. The development of algae (Harrison et al., 1988), invertebrates (Conner et al., 1988) and vertebrates (Bone et al., 1986; Falen et al., 1991) was studied.

The NMR microscopy was also applied to teratology (Kornguth et al., 1992). We have studied age-related modifications in thymus (Sbarbati et al., 1991b) and brown adipose tissue (Osculati et al., 1989, 1991). In this latter work, we found that ultrastructural examination of the brown fat at different ages showed three different patterns of adipocyte ultrastructure which were associated with different MRI patterns. Therefore, MRI can identify *in vivo* the prevalent type of adipocyte in the tissue providing information consistent with ultrastructural results. Jacobs and Fraser (1994) have recently used NMR microscopy to follow cell movements and lineages in developing frog embryos. A

single cell was injected at the 16-cell stage with a contrast agent and the labelled progeny cell was followed in three-dimensional images over several days. Smith et al. (1994) described techniques for the fixation, embedding, perfusion, and image acquisition of mouse embryos in normal conditions or treated with teratogens. In this report, the use of NMR microscopy for studying the embryonic vasculature and mapping the three-dimensional expression of genes was emphasized.

Experimental morphology

For its high spatial-temporal resolution, MRI has found a wide application in experimental morphology. In *in vivo* evaluations, the experimental paradigms are simplified with respect to traditional methods because each tissue is its own control. Usually, the structure is examined in a basal status and at different times after the induction of an event. For instance, a follow up analysis of transplanted tissues can be made (Dellagiacoma et al., 1992) or the volumes of spleen and lymph nodes of mice infected with retroviruses can be measured (Allegrini and Wachsmuth, 1990). Chronological sequences and blood-brain barrier permeability changes can be studied in brain edema induced by cryo-injury (Asato et al., 1983). The blood-brain barrier breakdown in MBP-specific T cell induced experimental allergic encephalomyelitis was studied by quantitative *in vivo* MRI (Namer et al., 1993). The most studied experimental pathology is surely cerebral ischemia. In a widely used animal model, the paradigm is based on a focal ischemia caused by a permanent or transitory occlusion of the middle cerebral artery in mammals followed by different treatments aimed to reduce the size of the ischemic lesion (Minematsu et al., 1993; Matsumoto et al., 1994). The extent of brain atrophy as determined by histological morphometry correlates well with the volume of edema measured by MRI (Weber et al., 1993). In other studies, animal models of global cerebral ischemia are used to investigate the casual connections between ischemia and NMR images (Bizzi et al., 1993). The evolution of a photochemically-induced cerebral thrombotic infarction was followed in rats during the first week after the insult by means of NMR-microimaging (Verlooy et al., 1993). MRI was also used to evaluate the effect of drugs on the size of intracerebral hemorrhages induced by stereotaxic microinfusion of collagenase in rats (Elger et al., 1994). In this type of experiment, before the introduction of MRI, the size of the lesion had been evaluated in histological sections. To date, in vivo MRI evaluation has replaced light microscopy in most cases because it permits a precise calculation of the effect of a given manipulation in the same animal which can be examined before and after treatment.

Cerebral ischemia is probably also the paradigm in which the correlation of MRI and light microscopy has been studied in most detail, and the correlation with immunocytochemical data is also available (DeWitt et al., 1986; Pierpaoli et al., 1993; Lo et al., 1994). In addition, in vivo examination is the first choice in paradigms of transitory ischemia when the reversibility of the lesions must be evaluated. In this type of paradigm, excellent results have been obtained by diffusion-weighted MRI, which is a technique of generating image contrast based on differences of restricted diffusion coefficents of water protons, a parameter sensitive to the presence of edema in the tissue (Moseley et al., 1990). Diffusion-weighted MRI can display regions of ischemic injury within minutes after onset (Minematsu et al., 1992). A technique of diffusion weighted fast spin-echo has recently been described for application of NMR microscopy. This technique permits a microscopic spatial resolution and accurate diffusion weighting (Beaulieu et al., 1992).

Drug testing

The foregoing statements demonstrate the utility of NMR technology in drug testing. It is evident from a review of the literature that the interest of the pharmaceutical industry in application of *in vivo* microscopy represents to date the main stimulus for the development of this technique. *In vivo*-MRI studies can parallel histological evaluation in testing the action of drugs and also increase the quality of toxicological data. To this purpose, histological studies are usually performed on some target tissues while other tissues are not examined.

Therefore, toxic actions of new drugs on tissues could be missed. MRI permits a rapid screening of the whole body of laboratory animals, thus obviating this problem. For example, using a paradigm including MRI we have recently described an effect of interferon on brown adipose tissue not detected in standard trials (Sbarbati et al., 1995b). A further example of the utility of MRI in drug evaluation in the gastroenteric apparatus is our recent morphofunctional study of rat gastric mucus secretion showing that MRI represents a sensitive screening test for pharmacological actions on the mucous layer (Sbarbati et al., 1995a). By conventional methods, information on the thickness of the gel mucous layer can only be obtained after complex manipulations (i.e. the gastric surface must be exposed, washed, mounted in a test tube, and examined). MRI permits an *in vivo* evaluation in the absence of surgical manipulations or washing of the mucosal surface. In addition, ^{1}H localized spectroscopy permits study of the time course of acid gastric secretion showing an increase in the water proton peak in the gastric lumen after injection of pentagastrin. Zhou et al. (1994) used surgically implanted RF coils to follow the development of liver pathology induced by bromobenzene exposure. The implanted coil acts not only to increase the quality of the signal but effectively localizes the same area of the tissue for sequential studies (Johnson et al., 1993). NMR microscopy can also be used to study inhibition of tumor cell proliferation in spheroids, spherical aggregates of cells with an outer proliferating layer, a

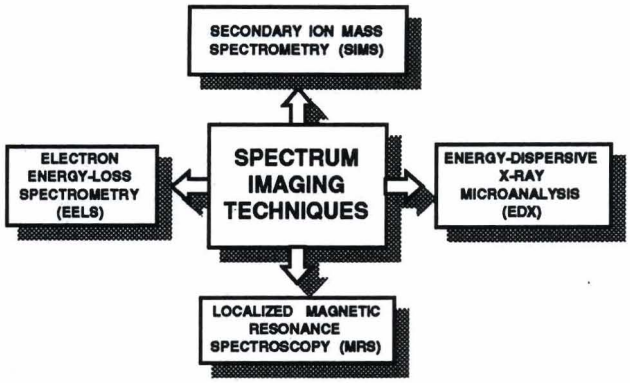

Fig. 2. Techniques providing a spatially-localized information on the chemical composition of specimens.

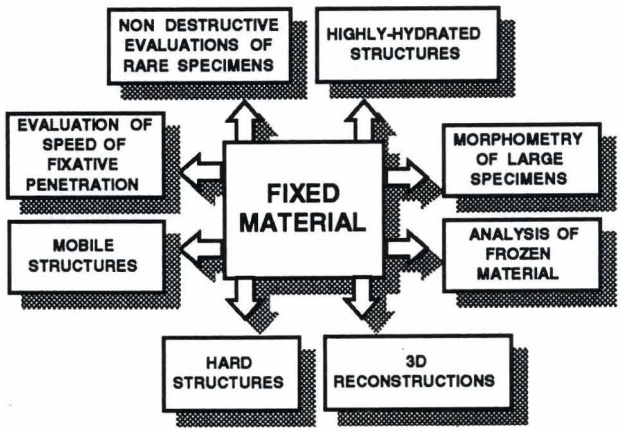

Fig. 4. Possible applications of MRI to fixed materials.

layer of quiescent cells, and an inner necrotic centre (Schiffenbauer et al., 1995).

Analytical morphology

The NMR signal directly originates from the chemistry of the biological object of interest and the derived information can be used to produce both spectra (giving compositional data, NMR spectroscopy) and images (giving spatial localization of structures, MRI). Furthermore, spectroscopic data can be obtained in a spatially resolved manner, even in the living organism (topical NMR spectroscopy). Therefore, NMR-technology is not merely a tool to obtain images but it also permits an evaluation of the biochemical composition of tissues. NMR images are density maps of different molecules containing ^{1}H, ^{13}C, ^{19}F, ^{23}NA, ^{31}P, or other atoms having a magnetic dipole moment. Therefore, by NMR technology we can acquire both spatial and spectral information in a combined imaging/spectroscopy experiment. As in other more used techniques (i.e X-ray electron probe microanalysis or electron spectroscopic imaging/electron energy loss spectroscopy) in NMR, the biochemical information can

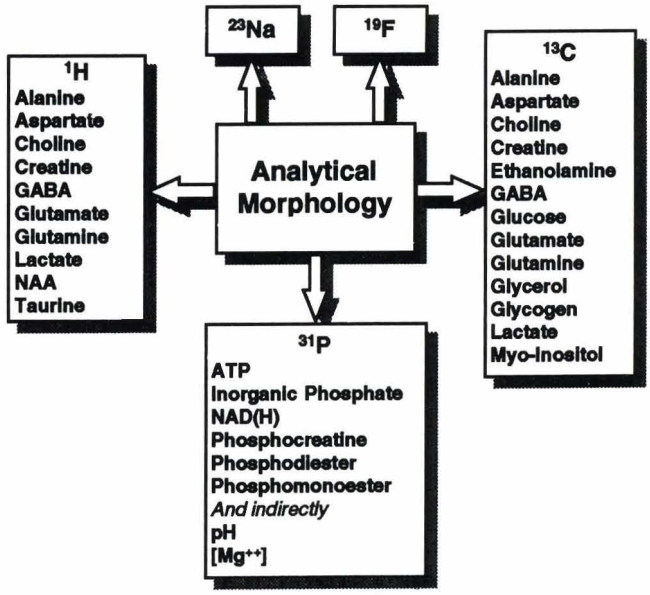

Fig. 3. Some representative chemical compounds that can be studied by localized NMR spectroscopy or selective metabolite imaging (collectively defined as analytical morphology).

be available in the form of a localized spectrum or of a density map (Fig. 2).

But with respect to other techniques, localized NMR spectroscopy is a technique particularly interesting because it can be performed in living animals providing a unique opportunity to study the *in vivo* metabolism of tissues (Taylor et al., 1983; Bottomley, 1989) (Fig. 3). For instance, MRI can be used to determine the spatial distribution of ^{23}Na in biological tissue (Wollf et al., 1990). Selective ^{31}P metabolite imaging has been obtained for high-energy phosphates (Jeneson et al., 1992). Spatial maps of metabolites such as glucose, sorbitol, and lactate have been obtained with submillimeter resolution by ^{13}C nuclear magnetic resonance (Cheng et al., 1991). These latter works demonstrate that while EDX, EELS, or SIMS can visualize only atoms, by NMR is possible to make a selective imaging of complex and biologically relevant metabolites.

Studies of fixed material

As previously illustrated, the main advantage of NMR microscopy over the traditional histological methods is the possibility of studying the living tissue. None the less, if only chemically-fixed or frozen material is available, the nondestructive NMR examination can provide useful information (Boyko et al., 1994) (Fig. 4). In addition, MRI can also be used to study cadavers instead of autoptic dissection. In fixed specimens motion artifacts are eliminated and the signal-to-noise ratio can be optimized by the utilization of a coil with maximum filling factor for the samples. The effects of formalin treatment on high field magnetic

resonance imaging has been calculated (Carvlin et al., 1989; Hedlund, 1991). The specimen can be studied avoiding dehydration, embedding and sectioning. This eliminates eventual artifacts caused by these treatments. In particular, in MRI, the sectioning is virtual and can be obtained in all planes (Suddarth and Johnson, 1991). This is useful in three-dimensional analysis and in morphometric evaluations of volumes of organs (Mayhew and Olsen, 1991) or internal cavity (Bendel and Eilam, 1992). MRI was applied to the study of normal and pathological human medulla oblungata (Vandersteen et al., 1994) or spinal cord (Lemaire et al., 1990). Hsu et al. (1994) studied chronic myocardial infarcts in formalin-fixed human autopsy hearts displaying the infarcted areas in three dimensions. MRI also permits measurement of the total amount of a tissue present in large specimens as well as in the living body (Seidell et al., 1990; Ross et al., 1991). Structures difficult to cut, or fluids can also be better evaluated by MRI than conventional techniques (Sbarbati et al., 1991c, 1992a,b; Henson et al., 1994).

Conclusion

MRI has become an increasingly appreciated diagnostic tool in medicine and it is probable that, in the future, its use in the field of microscopical morphology and cell biology will expand.

To date, this technology represents a challenge to the morphologist and needs interdisciplinary (i.e. physical, biochemical and physiological) competence. A review of the literature shows that MRI can be a precise and reliable tool to investigate the morphology and functional modifications of tissues *in vivo,* providing information consistent with histology. MRI has numerous advantages over conventional techniques: it is harmless to tissues; volume measurements in vivo could be useful for morphometric studies; the same tissue can be examined several times (e.g. at different ages); several organs can be examined at the same time; serial sections of relevant structures can be obtained in all planes; thereby allowing detailed reconstruction of the three-dimensional configuration of organs; motion with a tissue can be detected (Jenner et al., 1988); and subsequent histological and ultrastructural studies of a tissue are possible. Interesting possibilities have also been opened by the introduction of immunospecific NMR contrast agents produced coupling super-paramagnetic particles to monoclonal antibodies (Renshaw et al., 1986) or of contrast agents specific for structures visible at NMR microscopy (Kusaka et al., 1992). The main drawback (beside the cost of the basic instrumentation) is that resolution is relatively low in comparison with light microscopy. The analysis of the result is also difficult and needs interdisciplinary competence. In addition, MRI-histological methods are not yet well standardized. Therefore, in vivo MRI data must be evaluated with caution and histological controls are always required.

In conclusion, in our opinion, MRI is an interesting tool, complementary to other histological techniques and to date it cannot be ignored by microscopists. In morphological departments, the use of NMR-based technologies can result in an improved understanding of many issues and permit the execution of experimental paradigms not possible using conventional techniques alone.

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