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Fluorine magnetic resonance *in vivo*: A powerful tool in the study of drug distribution and metabolism

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Magnetic resonance (MR) provides an attractive non-invasive way of studying drug distribution *in vivo*. Widespread occurrence of fluorine in medicinal compounds, and its favourable MR properties, make it an effective probe for drug absorption, distribution, metabolism and excretion (ADME) studies. We discuss practicalities of detection and localization, and when ^{19}F MR would add value in a clinical trial, exemplified by deployments in oncology and psychiatry, where it is a practical way of demonstrating chronic brain accumulation directly. Limitations are emphasized to minimize failure risk, for example, inadequate sensitivity relative to tissue drug concentrations. The review anticipates increasing clinical ^{19}F MR as high field human scanners become widespread, and requirements to demonstrate mechanisms underlying clinical effects become more pressing.

In drug trials, confirming that the pharmacological agent has reached its target tissue is of inestimable importance. In preclinical studies this can be done by established ADME techniques, based on blood or tissue harvesting and subsequent drug and metabolite analysis. Conventionally, interrogation of tissue content usually involves terminal procedures that preclude longitudinal studies of accumulation, after acute and, especially, during chronic dosing, and washout, in which each subject is its own control. Plasma levels of compound often do not reflect concentrations in specific tissues, because of the presence of physicochemical barriers such as between blood and brain, and the activities of drug transporter systems which can both concentrate and deplete tissue relative to plasma. While indispensable to both the pharmacologist and regulator, this approach is less than perfect.

Clearly, in clinical trials, direct tissue (except blood) concentrations are even less accessible unless invasive biopsies are already integral to the study protocol. In practice, such procedures are a major complication and deterrent to volunteer recruitment and are unlikely to be included for the sole purpose of ADME measurement. Thus, any technology that can provide a reliable measure of tissue drug concentration, in the live subject and non-invasively, is extremely valuable.

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Non-invasive drug detection modalities

Positron emission tomography

In any practical sense these comprise only two techniques: positron emission tomography (PET) and nuclear magnetic resonance (NMR), usually referred to simply as magnetic resonance (MR). Coincidentally, the strengths and shortcomings [1,2] of each are almost complementary [3–6]. PET is very sensitive with limits of detection typically picomolar; this is of the same order as the effective tissue 'concentration' of numerous pharmacological target receptors, like those for neurotransmitters and hormones. This means that, for a high-affinity high-specificity PET ligand, it can be the case that the signal maps only the receptor occupancy, with minimal contribution from less-specific binding. Spatial resolution is typically in the order of a few millimetres.

For a drug development organization the main impediments for the exploitation of PET include the expensive facilities (infrastructure such as the cyclotron together with supporting personnel) required to generate short half-life positron emitting isotopes (^{18}F about 2 h, ^{11}C about 20 min) and the need to devise rapid radiochemical synthesis, purification and analytical procedures for new chemical entities (NCEs) which are consistent with these half-lives. In practice, devising a synthetic route for labelling an NCE can take a year or more, requires teams of specialist radiochemists, and is often incompatible with the demanding timelines typical of early drug development. Also, the PET isotope with which it is easiest to work is the comparatively long-lived ^{18}F [7], so only molecules containing fluorine can be observed directly. As a result, many receptor occupancy studies on compounds with specific pharmacology rely on competition against a small number of known agonists or antagonists of the same class for which PET radiosyntheses are well established [8–10]. Rigorous interpretation of a competition study also requires complex modelling of distribution between plasma, receptors, and other compartments, and assumptions about the metabolic processing of subpharmacological tracer doses. For example, when active metabolites are formed *in vivo*, as is the case with many cancer therapeutics, precise modelling from PET data may become impossible [11]. Also, because of the short half-lives it is impossible to measure slow accumulation of a chronically administered compound directly. It is a regulatory requirement to perform radiotoxicity studies on a new radioligand before administration to volunteers [12] and radiotoxicity issues limit the repeatability of PET exams in volunteers.

Having said which, when all these challenges have been surmounted, there are few more convincing demonstrations of drug distribution than PET images of a labelled tracer in a living subject. Such pictures are powerful scientific tools and their value to a marketing organization should never be underestimated. Furthermore, recently published FDA guidance on exploratory IND (investigational new drug) studies offers a regulatory framework to expedite studies at subpharmacological doses (including novel PET tracers) before conventional Phase 1 studies [13]. Where acute brain penetration or receptor occupancy are issues, interrogation by PET should always be considered.

Magnetic resonance in drug tracking

MR, on the other hand, does not use ionizing radiation so volunteers can be scanned as frequently and as often as necessary and ADME can be followed over any necessary period of time. Volunteer studies with INDs can be performed under the same regulatory and ethical approvals as any other clinical trial, provided scanning is consistent with the protocol and tolerated by volunteers. Another factor which is important in the design of a clinical trial involving MR is that no special formulation technology needs to be developed or applied for the preparation of the test substance in a relevant dosage form.

The major drawbacks of MR arise from its inherent insensitivity, and the fact that isotopes of most of the elements of pharmaceutical relevance, except ^{19}F, have obtrusive natural biological background signals. This has meant that, hitherto, the major use of MR in drug trials has been to provide an imaging readout of anatomical or functional biomarkers, such as vascular plaque burden, stroke or myocardial infarct volume, cartilage erosion, or neurological activity, with or without drug intervention [14,15]. Indeed, a search under 'MRI' on the U.S. National Institutes of Health clinical trials registry website [16] currently retrieves many hundreds of studies using MR as a biomarker readout. MR spectroscopy [17] is less widely used, but a search of the same website still shows over a hundred studies using MRS to provide metabolic biomarkers.

^{19}F as a favourable probe

Favourable intrinsic NMR properties – 100% natural abundance, spin-, high γ, all predisposing to sensitive signal generation – confer on ^{19}F MR high potential for non-invasive measurements of drug ADME [18], especially with the proliferation of high field clinical scanners. One obvious limitation is that the compound of interest must contain fluorine, but the element is widely represented in marketed compounds of most pharmacological classes and is favoured in discovery medicinal chemistry, so this factor is not as restrictive as it might first appear [19].

Drug–macromolecule interactions

Another complication peculiar to magnetic resonance, is that non-specific drug binding to 'non-target' biological macromolecules and biomembranes, which to a lesser or greater extent occurs for most pharmaceuticals, increases signal linewidth, because of effects on effective molecular tumbling rate. This decreases the fundamental NMR property T_2 (the 'spin–spin' or 'transverse' relaxation time) which has the practical effect of 'smearing' the useable NMR signal over a wider frequency bandwidth, which compromises signal detection. In the worst case, drug signals can be broadened beyond detection, giving rise to pessimistic or negative conclusions about access to the tissue of interest. Fortunately, protein-binding propensity can be predicted from the physical chemistry of the compound which is often measured directly as part of a development package; extreme lipophilicity or high protein binding in any of these tests should act as a caution.

Detection levels

The limited sensitivity of MR means that, in favourable circumstances, tissue levels of a drug or metabolite of interest need to be at least a few micromolar and the best spatial resolution that can be hoped for is currently limited to centimetres. This means that detected signal is unlikely to reflect compounds occupying their

pharmacological receptors, which are usually at very low concentration (in terms of receptor per millilitre of tissue), but rather is a measure of the total tissue content. Nevertheless, the attraction of being able to measure organ concentrations without invasive, stressful biopsy procedures in clinical trials is compelling, especially when there is reason to expect that tissue concentrations differ markedly from plasma levels.

Figure 1 shows the structures of compounds at the centre of the literature on direct *in vivo* detection by ^{19}F magnetic resonance. They span a wide range of chemical classes and therapeutic targets, showing that the approach is general across the pharmacological spectrum. Nevertheless studies of brain-penetrant psychoactive compounds, and anticancer cytotoxics and antimetabolites, do predominate, probably for a combination of reasons. In the CNS case, compounds can accumulate slowly and ultimately reach appreciable brain levels amenable to MR detection. As far as the anticancer agents are concerned, therapeutic doses are high and compounds have a tendency to be hydrophilic, so MR detection is often straightforward.

Practicalities

There are excellent web resources on the websites of the Society for Magnetic Resonance in Medicine (ISMRM) [20] and the European Society for Magnetic Resonance in Medicine and Biology (ESMRMB) [21].

Signal localization and acquisition

In principle, there are multiple MR approaches available to the investigator to achieve tissue localization. The simplest is to use a radiofrequency (RF) coil to detect signal from a volume prescribed solely by the RF-coil size and geometry. More sophisticated acquisition methodologies are available to provide localization in single

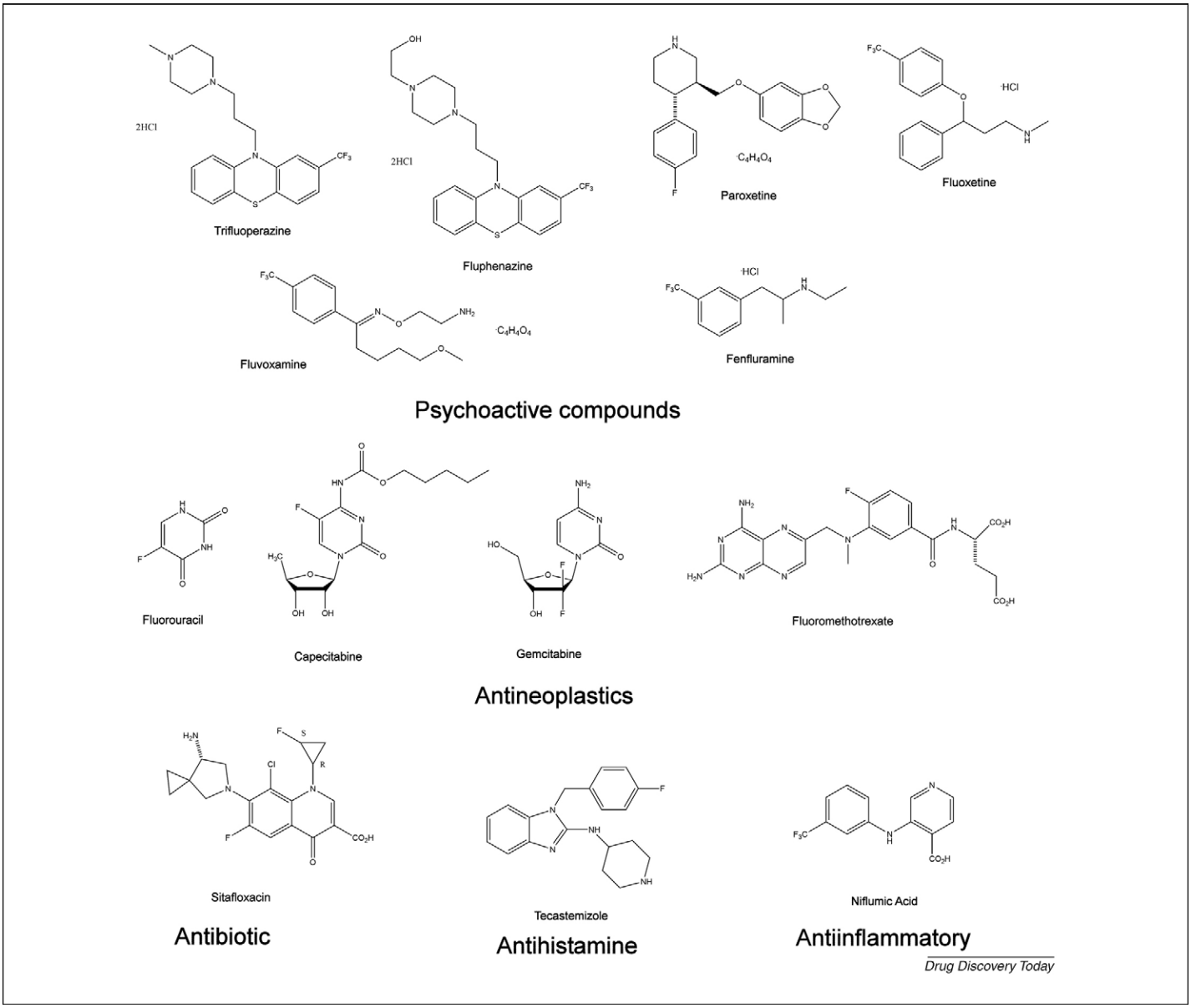

FIGURE 1

Chemical structures of pharmacological agents studied by *in vivo* MR.

geometrically defined volumes (for example, a 2 cm × 2 cm × 2 cm cube) or multiple volume elements (voxels) in a plane or volume which have the potential to provide metabolite maps. For reasons of simplicity and sensitivity, the first approach is generally favoured for ^{19}F MRS. Typically, a signal is collected from the entire sensitive volume of the RF-coil. In brain studies this is often a volume resonator, which acquires signal from the entire head. In studies of abdominal organs or peripheral structures, the investigation is most likely to be performed with a 'surface coil' detector placed over the site of interest, such as the liver or a solid tumour.

This acquisition methodology has an attractive simplicity; ^{19}F magnetization is excited with a single RF-pulse, detected, and this process repeated and the collected signals averaged. In addition to the localization scheme, appropriate selection of certain MR acquisition parameters is fundamental to success. One key MR parameter is the repetition time (TR). This parameter will dictate signal-to-noise ratio and acquisition time and, therefore, optimization is important. Choice of TR will be dictated by the intrinsic NMR parameter T_1 of ^{19}F in the tissue of interest and is typically a few hundred milliseconds. Because signal is being collected from a comparatively large volume, acceptable data can usually be collected in a few minutes. This gives a time resolution that is generally quite sufficient to define ADME processes in acceptable detail. A drawback of surface coil acquisition is that it is not spatially uniform, and signal falls off with distance from the plane of the coil.

^{19}F and ^{1}H (which is the isotope that is detected in all commercial clinical scanners), resonate very close to each other (60 and 64 MHz respectively, in a 'workhorse' clinical 1.5 T magnetic field scanner). This means that it can be straightforward for a competent bioengineering workshop to modify a clinical ^{1}H detector coil to resonate at ^{19}F frequency and, therefore, detect ^{19}F instead of ^{1}H, or in addition to ^{1}H if the coil can be double tuned. The latter capability offers the possibility of co-registering an ^{19}F drug distribution map with a high resolution ^{1}H anatomical image, and even other MRI-based acquisitions, such as a functional MR image (fMRI) showing the physiological consequences of pharmacological activity.

Magnetic field strength

Scanners operating at fixed magnetic field strengths higher than the standard 1.5 T diagnostic MRI machine are proliferating in laboratory and clinical settings. Clinical scanners are now widely available at 3 T, and manufacturers are producing even higher field machines (including 7 T) for specialized clinical applications, such as head imaging. The MR sensitivity increases approximately linearly with magnetic field strength, so lower limits of detection and quantification should go down as investigations use higher field scanners. In addition to increasing field strength, more sophisticated spectroscopic acquisition schemes based on the use of the scanners, pulsed magnetic field gradients, such as voxel-selective spectroscopy techniques like PRESS, and chemical shift imaging (CSI) techniques [22], are likely to become more widely available, making metabolic imaging approaches more common. Increased field strength is not, theoretically, without some challenges; higher field strength equates to higher signal frequency, requirements for more sophisticated coil design and tuning, reduced signal penetrancy, and increased RF power deposition. Also there is currently discussion of the hazards, to subject and operator, of exposure to high magnetic fields, and moves to regulate this exposure [23].

The structure of the molecule

The most favourable compounds, as far as sensitivity is concerned, are those containing multiple chemically equivalent fluorine atoms, such as in trifluoromethyl (CF_3) groups, which contribute to increased signal *per* molecule. In such cases, concentrations averaged over an entire tissue of interest, such as the whole brain, greater than micromoles/litre are often detectable in practical blocks of time. Even reports of monofluorinated paroxetine [24] implies successful detection of ca. 2 μmoles/l levels, so the presence of only a single fluorine in a compound of interest should not necessarily deter investigation with ^{19}F MRS. Estimation of drug concentration can be achieved by reference to signal intensity from samples – 'phantoms' – of known concentration placed next to the volume of interest, but within the sensitive volume of the coil. Alternatively, ^{1}H measures of tissue water during the same examination can be used as an internal reference from which ^{19}F MRS drug concentrations can also be estimated.

The value of preclinical studies

Before committing resources to a clinical trial in volunteers, the feasibility of detecting therapeutic levels of compound should ideally be confirmed in preclinical species if the facilities for doing so are available [25]. This requires the drug development organization to have access to preclinical scanners and support infrastructure, such as facilities and personnel for housing animals, and dosing and conducting scanning to the animal welfare standards demanded by institutional and national regulations. It is a fact that currently these facilities are scarce outside major academic institutions and large pharmaceutical companies, where they are usually heavily oversubscribed. At the very least, preclinical (and clinical to the extent that they may be available) ADME data should be consulted to establish whether drug levels in the target tissues are likely to reach MR-detectable (i.e. micromolar or higher) levels. Should this not be the case, it makes no sense to invest in an MR study. Even if ADME suggests MR-detectable drug levels, macromolecular interactions may still render drug signals unobservably broad. While this is an imponderable that can only be answered with total confidence by an *in vivo* experiment, drug–macromolecule binding does tend to increase with drug lipophilicity, so a highly lipophilic compound is unlikely to be a good candidate for MRS, even at high tissue concentrations.

The future is epitomized in the study of Bolo *et al.* [26] on brain distribution of the selective serotonin reuptake inhibitor (SSRI) antidepressants, fluvoxamine and fluoxetine, which highlights the advantages of working at high (3 T) field strength as shown in Figure 2. The top left false coloured image shows the distribution of the antidepressant fluvoxamine in a volunteer's brain, acquired using spectroscopic imaging that selects only the signal of the target compound for construction of the image. The top right panel shows a spectroscopic image, tuned to collect signal only from the trifluoroethanol in an external phantom; its signal has been used as a reference, of known concentration, to estimate brain levels. Although spatial resolution is low, the example presages the type of drug distribution image we can expect from MR in

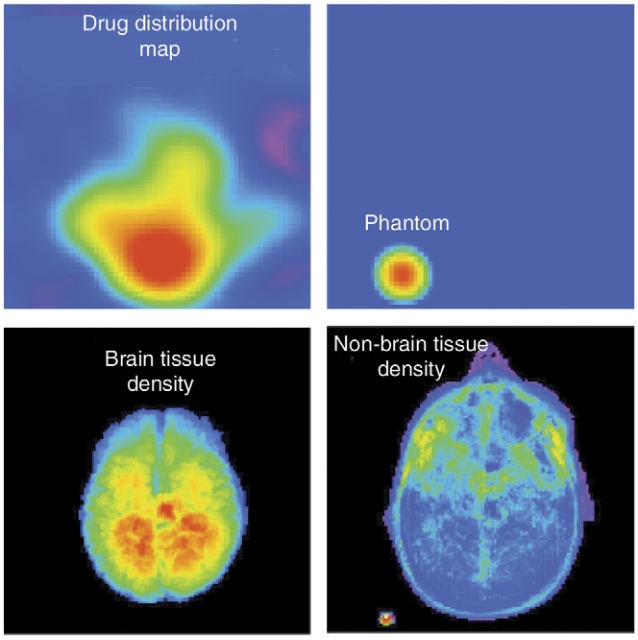

FIGURE 2

The distribution of fluvoxamine in the brain of a volunteer shown by ^{19}F MR chemical shift spectroscopic imaging at 3 T (top left), and of an externally positioned trifluoroethanol phantom used for quantification (top right). The bottom panels reflect the density of brain (left) and non-brain (right) tissue in the slice of the ^{19}F image, calculated from standard proton three-dimensional MRI (adapted from Bolo *et al.* [26] and reproduced with permission from Nature Publishing Group).

a future of higher field strengths and refined detector, acquisition and image processing technology.

Studies of psychoactive agents in brain

A compelling area of application of *in vivo* ^{19}F MR is in the detection and quantification of psychiatric compounds in the brain [17,27]. A principal driver of work in this area is the fact that accumulation of agents in the brain can be much slower than the attainment of steady-state concentrations in plasma (which can be measured much more easily). In a clinical trial this can predispose to the false negative conclusion, that a compound is inactive, when in fact it has simply not yet reached its effective concentration in the brain. By the same token, eventual steady-state brain levels can exceed plasma levels by orders of magnitude, because of trapping of compound on account of physicochemical, or other, phenomena. Again, side effects related to compound withdrawal relate to brain, and not plasma, levels so it is the rate at which a compound clears from the brain that is important for our understanding. There are numerous studies in the literature on patients receiving chronic antidepressant or antipsychotic treatment, in which the study focus has been brain quantification, correlation with clinical effect and the relationship between brain and plasma levels. Some are discussed below.

For instance the brain and serum pharmacokinetics of the *R*-enantiomer, and the racemate, of the antidepressant selective serotonin reuptake inhibitor (SSRI), fluoxetine, [28] have been compared [19]. The *S*-enantiomer (present in the racemate) happens to be a potent inhibitor of drug-metabolizing cytochrome P450 enzymes, so its half-life and that of its des-methyl metabolite, norfluoxetine, is much longer than that of the R enantiomer; MR is a sensitive indicator of this discrepancy when volunteers are dosed with the different forms (note that in this, and all other reports, MR is not claimed to distinguish enantiomeric pairs spectroscopically, although this would be possible in principle if each enantiomer interacted differently with macromolecules playing the role of the 'chiral shift reagents' used in chemical NMR analysis). Brain concentrations in volunteer patients on chronic doses were shown by ^{19}F MR spectroscopy to be about an order of magnitude higher than plasma levels.

Whole-brain ^{19}F MRS has been used in paediatric patients to correlate dose of fluoxetine and another SSRI antidepressant fluvoxamine, with brain levels after several weeks dosing [29]. Possible effects on signal detectability of fluoxetine binding to macromolecules are studied using 'magnetization transfer' (MT); by measuring the extent of drug signal lost when the very broad putative macromolecule-bound signal is removed by an off-resonance RF pulse, the fraction of drug which is bound can be estimated. In addition, they are able to measure brain levels of both compounds in the micromolar range, which correlate with dose. MT suggests the existence of a ^{19}F MRS 'invisible' pool of bound fluoxetine in human brain; percent MT signal losses are between 7% and 20% [30] and a negative correlation between MT and brain fluoxetine levels implies saturable binding. These studies exemplify the power of MRS to elucidate more complex aspects of drug compartmentalization. The existence of a pool of macromolecule-bound fluoxetine not detected by direct ^{19}F MRS was first suggested [31] by a comparison between human brain measurements and *ex vivo* studies at autopsy in which the latter were somewhat higher than the MRS estimates.

Henry *et al.* [24] correlate clearance of chronically administered paroxetine or fluoxetine from brain after placebo substitution with the frequency of adverse withdrawal-related events in patients. In a study at 3 T, Bolo *et al.* [26] examined brain PK and tissue distribution of fluvoxamine and fluoxetine. Brain levels of fluvoxamine and fluoxetine are in the order of tens of micromolar and about an order of magnitude higher than plasma, although they correlate between the two compartments. This study has already been alluded to as exemplifying the benefits of high field and the access that this will give investigators to a true imaging output of drug distribution.

Strauss et al. [32] measure whole brain fluvoxamine levels after cessation of treatment and found mean brain elimination half-lives (58 h) much longer than plasma (26 h) after several weeks of continuous dosing. Strauss *et al.* [33] measure acute and steady-state fluvoxamine levels in brain. There was no detectable ^{19}F signal after a 100 mg bolus dose, but the compound became observable at steady-state concentrations between about 3 and 40 μmoles/l, with corresponding plasma concentrations about an order of magnitude lower. In social phobia patients, brain concentrations of fluoxetine of about 10 μmoles/l were detectable and correlated with clinical response [34].

In an early demonstration of the practicability and usefulness of ^{19}F MRS, concentrations of fluoxetine and the neuroleptics, trifluoperazine [35] and fluphenazine were estimated [36], in the

brains of patients chronically receiving clinical doses; they were detectable at levels of tens to hundreds of micromoles per litre.

Preclinical studies

If an investigator has access to a preclinical ^{19}F MRS capability, experiments in suitable species can provide vital evidence that the approach will be worth pursuing in clinical ADME studies. Data acquisition volumes of interest are generally smaller than in clinical studies, but the sensitivity loss this leads to is generally, at least partially, offset by the fact that the preclinical studies are likely to be conducted at higher field strengths, typically 4.7, 7 or even 9.4 T. In monkeys, the anorectic compound, dexfenfluramine, and its metabolite, D-norfenfluramine, were observable [37] at brain levels around a hundred micromoles per litre after 5 days of dosing. Comparison with *ex vivo* chromatography data supported the conclusion that all drug in the brain was MR visible. In rats, [38] fluphenazine was visible in the brain after three weeks dosing, with *in vivo* levels estimated as several tens of micromoles per litre. The same group [39] studied trifluoperazine in rat and fluphenazine in rat and human brain, the latter at 3 T. In the TFP experiment (single dose intravenously), the drug was visible immediately after bolus administration and for up to five hours thereafter.

Drug accumulation and metabolism in cancer

In chemotherapy of solid cancers, maximizing the quantity of agent accessing the lesion, relative to systemic levels, obviously increases therapeutic response and minimizes unwanted side effects. Responses to given drug regimes can be highly variable between patients because of the variety of drug influx and retention mechanisms, metabolic pathways, and efflux pumps, at the disposal of the tumour cell. In a clinical trial, ^{19}F MR could potentially help segregate patients who take up a compound, and respond, from those who do not, enabling preselection into early development studies, and optimization of individual treatment regimes. The fluoropyrimidines, such as the antimetabolite 5-fluorouracil (5-FU) and its analogues and derivatives, have been extensively studied. They are currently first line treatments in cancers of the colon-rectum, head and neck, oesophagus, stomach, and some forms of breast cancer. MR is particularly useful in differentiating patients who are 'trappers' [40], who accumulate the drug at the target site, from non-trappers, who do not and are therefore unlikely to respond. It would clearly be useful in testing the treatments which involve increasing trapping, pharmacologically (e.g. with methotrexate), or physiologically (e.g. with carbogen breathing). The use of clinical and preclinical ^{19}F MR to study ADME of anticancer compounds has been extensively reviewed [4,6,41–43].

Figure 3 typifies the use of ^{19}F MR spectroscopy in the study of anticancer drug distribution and metabolism [44]. It shows a spectrum acquired in only a few minutes from the liver of a patient given a bolus dose of 5FU. Not only is the parent drug clearly visible and quantifiable, but so also are metabolites like fluoronucleotides (which can be toxic) and fluoro-β-alanine. A technique used to increase tumour uptake of drugs is to switch patients temporarily to breathing carbogen; its effects on 5FU uptake and on the distribution of its metabolites is clearly shown by ^{19}F MR.

Schlemmer *et al*. [45] study 5FU PK in head and neck carcinoma during combination radiochemotherapy, using a surface coil placed over the tumour. The ^{19}F MRS enables hypotheses about possible synergies between 5FU treatment and radiotherapy in enhancing the delivery of the former into the target tissue to be made.

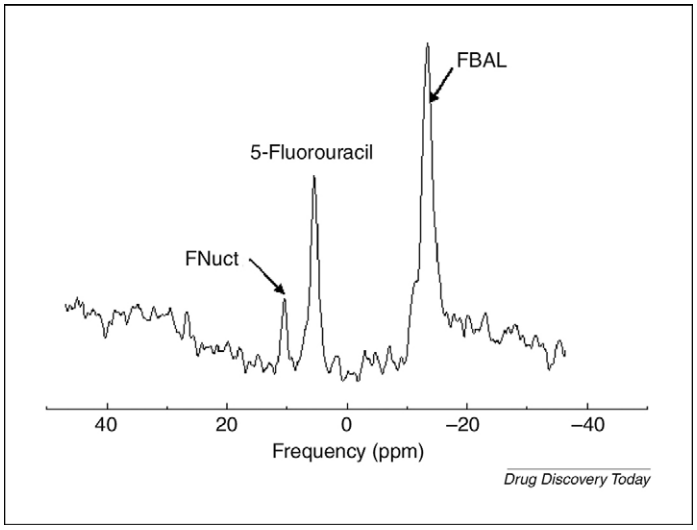

FIGURE 3

5-Fluorouracil and its metabolites detected *in vivo* by ^{19}F MR spectroscopy (adapted from Griffiths *et al.* [44] and reproduced with permission from Taylor and Francis http://www.informaworld.com/actaoncologica). The spectrum, from the liver of a patient who had just received a 5-FU bolus dose, took 16 min to acquire with a surface coil.

Preclinical studies

Much insight into the mechanism of action of anticancer drugs has been gained using ^{19}F MRS of experimental therapies in preclinical models based on implanted tumours, which lend themselves well to surface coil observation. Thus, the ADME of capecitabine, a prodrug of 5FU, has been investigated in tumour bearing nude mice [46] and related to thymidine kinase activity, which is a good predictor of clinical response. Signals from the parent compound and metabolites 5′-deoxy-5-fluorocytidine (5DFCR) and 5′-deoxy-5-fluorothymidine (5DFTR) are detected in 10 min acquisition blocks enabling detailed PK profiles to be established. A monofluorinated analogue of the widely used anticancer agent methotrexate, fluoromethotrexate (FMTX) [47] gives a good indication of methotrexate ADME in a mouse tumour model. Detection of submillimolar levels of drug in the tumour can be achieved at a field strength of 4.7 T. The same group [48] follow up with a study correlating uptake and retention of the same compound in three nude mouse models (human sarcoma xenografts) of known differing sensitivity to MTX. Again, using a surface coil approach and 30 min time windows, gemcitabine ADME [49] has been characterized in nude mouse tumours. A useful application is to study the effects of treatments designed to increase drug uptake into tumours, such as [50] carbogen breathing which is thought to transiently open tumour vessels.

Quantitation and metabolism in the liver and other organs

Because it is an important location for drug accumulation and metabolism, and a significant site of side effects, the liver has received much attention from spectroscopists. The activity of

many orally administered compounds is affected by so-called 'first pass' metabolism, because of the compound encountering the drug metabolizing machinery of the liver as it is absorbed from the gastrointestinal tract and traverses the hepatic portal circulation. This means that systemic levels of the active parent compound can be lower than required for therapeutic effect, and that concentrations of metabolites, which may be accompanied by unwanted secondary effects, can be unexpectedly high. Also, pharmaceutical development of novel controlled release formulations, designed to liberate active compound slowly over hours or even longer periods, is currently an area of intensive activity as a successful new formulation leading to a 'product line extension' can increase the value and extend the profitable life of a drug substance. Tracking the absorption and metabolic fate of compounds in the context of these new devices is likely to be a significant new area of *in vivo* MR application [51].

In a validation study for the use of ^{19}F MRS to quantify the experimental antihistamine tecastemizole (an experimental once-a-day treatment for allergic rhinitis) in heart and liver at 4 T, Schneider *et al.* [52] found a limit of detection of ca. 20 μmoles/l in volunteers. They compared their results with Bolo *et al.* [53] on the detection of fluoxetine and fluvoxamine (ca. 40 μmoles/l) in peripheral bone marrow and with Bilecen *et al.* [54] data on the trifluoromethylated non-steroidal anti-inflammatory drug (NSAID) niflumic acid. In the latter study, in humans, first pass metabolism produced two signals in the liver, parent compound and the metabolite 4′-hydroxyniflumic acid. Micromolar levels of compound could be detected in time windows of several minutes. The fluoroquinolone antibiotic sitafloxacin is visible in human liver after chronic dosing. Mean levels detected were tens of micromoles/litre, considerably higher than in plasma, with a detection threshold of about 5 micromoles/litre [55].

In human liver studies, van Laarhoven *et al.* [56,57] monitor the metabolism of capecitabine at 1.5 and 3 T, in patients with metastatic colorectal cancer. These studies contain telling illustrations of the benefits for both signal-to-noise and spectral resolution of the transition from moderate (1.5 T) to high (3 T) magnetic field. Signal-to-noise increases several fold, and more metabolites, such as bile acid conjugates, become detectable and resolvable. Ikehira *et al.* [58] use a surface coil approach to study metabolism of 5FU based compounds in liver with signal becoming detectable immediately after oral administration. Sassa *et al.* [59] look at the distribution of the monofluorophenyl-containing antipsychotic haloperidol decanoate (used as a slow release sesame oil-based maintenance treatment) after intramuscular injection at 1.5 T.

Future developments and applications

Future uses of ^{19}F MR in non-invasive drug ADME studies are going to be driven by the increasing need, both scientific and regulatory, to demonstrate understanding of pharmacological mechanisms underlying clinical outcomes and side effects. Proving that a drug has reached therapeutic levels in target tissues, and understanding its processing to active, inactive, or competitive, metabolites, is of vital importance. The increasing cost of drug development and the challenges of developing new medicines, especially those acting by unprecedented mechanisms, are well known. In a drug development programme, early demonstration of a compound's unsuitability on grounds of poor bioavailability, is of immense commercial value. In this, the presence of fluorine in a compound provides the clinical scientist with a built-in probe which should be exploited to as great an extent as possible. Practical assistance will come from the proliferation of high field clinical instrumentation, supported by the constant advances being made in hardware such as the advent of useable cryoelectronic preamplifiers and other components.

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