Journal of Magnetic Resonance 306 (2019) 145–149

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Imaging molecules

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ARTICLE INFO

Article history:
Received 1 April 2019
Revised 27 May 2019
Accepted 8 July 2019
Available online 9 July 2019

Keywords:
Molecular imaging
MRI
CEST
Spectroscopy
MRSI
MTC
ihMT
qMT

ABSTRACT

Molecular imaging using MRI is gaining momentum. While sensitivity of MR is limited compared to other molecular imaging modalities, the molecular specificity is high in comparison. Moreover, MRI offers contrast based on multitude of processes and scales, from intramolecular relaxation pathways to water diffusion. Living tissue offers abundance of potential molecular targets of interest in biology and medicine. In this short perspective we focus on some direct and indirect methods to visualize endogenous molecules. We briefly discuss Spectroscopic Imaging (MRSI), Chemical Exchange Saturation Transfer (CEST) and Magnetization Transfer Contrast (MTC). Imaging molecules with MRI is part of the larger universe of imaging methods. Moreover, it is part of ever increasing pool of data combining imaging with other modalities, biology and patient outcomes.

Molecular imaging is "non-invasive visualization of biochemical events at the cellular and molecular level..." [1]. Many imaging modalities fall under this umbrella, such as PET and optical imaging, and Magnetic Resonance (MR). However, as a molecular imaging modality MRI suffers from inherently low sensitivity [1], compensated by high molecular specificity. In the last decade molecular MRI has undergone many advances. In this short perspective, I will focus on the attempts to visualize directly or indirectly specific *endogenous* molecules or molecular assemblies in-vivo using RF or gradient pulses and without exogenous substances.

Magnetic Resonance provides the unique ability to identify and characterize molecules based on their chemical environment, starting with chemical shift information. Moreover, it provides unique tools to study dynamic processes, such as chemical exchange, chemical reactions and molecular rearrangements. Live tissue is incredibly complex and, thus, is full of potential molecular MR targets. One of the fascinating questions, in my view, is how to utilize the unique capabilities of MR to study molecules and molecular properties in vivo, using these intrinsic "contrast agents". And there are plenty of targets: metabolites, lipids, large molecular aggregates. All of them can potentially be targeted. However, can the detection be sensitive, specific and achievable within realistic time frame? And, ultimately, can any of the methods provide meaningful information about biology, pathology or have meaningful and positive impact on patient care?

Direct visualization, such as spectroscopy or spectroscopic imaging (MRSI) still offers the most direct window into specific molecules or metabolites (Fig. 2). Advancement of high and ultra-high fields technology (>4T) allows for improved SNR [2] and offers uncontestable advantages for proton and non-proton MRI, such as 13C, 31P and 23Na [3–5]. This, in turn, allows identification and quantification of numerous metabolites *in-vivo* in preclinical and clinical studies of various diseases [6]. Such studies allow direct quantitative measurements of metabolic events, e.g. creatine kinase rate [7] or redox state [8], to name just a few (see Fig. 2 for examples). At the same time, the introduction and advancement of Parallel Imaging, Compressed Sensing, as well as other rapid acquisition methods may allow orders of magnitude reduction in acquisition times for MRSI and the creation of true metabolite maps [5,9,10]. These advances open a road to increased usage and studies in animals and humans. However, without dramatically increasing intrinsic sensitivity (i.e. hyperpolarization) and widespread availability of high field scanners, application of MRSI may still require prohibitively long acquisition times for full translation into the clinic. Parallel technical advances made direct observation of specific molecule possible and more accessible, even at the lower, clinically accessible, fields (3 T). For example, spectral

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https://doi.org/10.1016/j.jmr.2019.07.022
1090-7807/

editing allows non-invasive determination of IDH mutation status in GBM tumors, which may have positive impact on clinical management of patients [11]. In a different development, advancement of ultra-short detection schemes may allow direct visualization of myelin, which is typically prohibited by its short T_2 [12]. Further advancement of technology (higher fields, smart sampling, shorter TE's, etc. . .) will push for broader and broader utilization of these methods in pre-clinical and clinical imaging.

Indirect observation of molecules offers an attractive route to enhance otherwise unobservable signals (schematically depicted in Fig. 1 and inlays in Figs. 2 and 3). One of the methods gaining widespread recognition is Chemical Exchange Saturation Transfer [13–17] (CEST). By selectively saturating a specific off-resonance frequency and subsequent transfer of the saturation to water, the indirect detection of the whole molecule (or group of molecules) can be achieved [18,19]. CEST provides signal amplification, allowing detection of moieties in the mM concentration ranges, that are not detectible otherwise (vs 110 M water concentration). CEST is a subset of a large family of Magnetization Transfer (MT) experiments, where magnetization prepared via RF (typically saturation pulses) is transferred to water for observation. Another member of this family is Magnetization Transfer Contrast (MTC) that utilizes the semi-solid properties of macromolecular pool [20]. In CEST and MTC the transfer occurs via chemical exchange, dipolar relaxation or a combination of the two [21]. One of the caveats in CEST, to achieve specificity, is that the exchanging group needs to tumble fast enough so that its spectral line is sufficiently narrow. Second, for the most efficient transfer the exchange needs to be in slow or intermediate rate (as defined by NMR scale), so that a separate line could be observed. Another, closely related method to CEST, is T_{1rho} which may offer some advantages for detection of chemical groups in fast and intermediate exchanging regimes [22]. Due to the nature of the transfer, CEST depends on both, molecule concentration and on chemical exchange rate.

Since the CEST experiment selectively "labels" a specific chemical group, the whole molecule could, in theory, be specifically detected. While ultimate specificity may remain elusive in-vivo, a contrast "weighted" by a specific molecule can be generated. One of the first examples was CEST from amide protons (Amide Proton Transfer (APT) [23,24]). Since the protein backbone offers an abundance of relatively fast tumbling amide groups and since their exchange rate is pH dependent, APT was proposed as a direct marker of the pH and protein content. The dual dependence of CEST provides a simultaneous advantage and disadvantage. On the one hand, CEST is a potential direct pH marker in tissue [24]. On the other hand, the dual dependence complicates molecular specificity and quantification and makes challenging the determination of the molecular concentration without the influence of the exchange rate [25,26]. In addition to APT, abundance of endogenous CEST molecular targets had been proposed and successfully observed in-vivo: creatine [27], glycosaminoglicans [28], myo-Inositol [29], etc., (Fig. 3 depictcs few examples). While the emerging body of work is impressive, specificity of CEST contrast to a molecule is often uncertain. First, even if a chemical shift difference of a specific chemical group from water is known from ex-vivo or phantom studies, in-vivo that frequency may change, due to the environmental influence (pH, temperature, etc.). Second, different molecules can possess same chemical group (e.g. –OH) leading to overlapping spectral lines. Third, the saturation RF bandwidth is not infinitely sharp and inevitably will saturate the exchanging moiety as well as surrounding spectral areas and underlying, broader lines leading to unwanted MTC. This unwanted MTC from a semi-solid pool may obscure the CEST signal. Finally, there is inevitable direct water saturation complicating detection of groups with chemical shift close to water.

Number of innovating methods has been proposed to circumvent some of these limitations. For example, there is an optimal B_1 intensity for each exchange rate [30]. Thus, CEST imaging could be made more sensitive to a particular group over others by carefully optimizing RF saturation train, see e.g. Ref. [31]. Additional methods, not relying on saturation were developed to enhance specificity. For example FLEX, employs not a saturation pulse, but a number of excitation pulses with gaps (the so-called labeling modules), leading to frequency transfer [32]. Utilization of methods such as Variable Delay Multi-Pulse trains (VDMP-CEST [33]) or Transfer Rate Edited Experiment for the Selective Detection of Chemical Exchange via Saturation Transfer (TRE-CEST [34]) allows better MTC suppression and tuning for a specific transfer rate. Dual frequency saturation can be employed to better remove interfering broad MT "background" [35,36].

In the methods relying on saturation, a promising approach for improved CEST specificity involves high and ultra-high fields. Since the increase in field strength increases the spectral separation between lines, some exchanging moieties may cross into the slow exchange regime. Thus, different chemical shifts and molecules could be separated better. The advantages of high fields, however, come with a price: RF homogeneity problems and potential SAR limitations in humans. Moreover, currently human high field machines are limited to a handful of advanced centers, thus restricting adaptation and dissemination into clinic. Nevertheless, the information gained at the high field could be used and translated to lower fields, which are more accessible. Such an approach would allow advantages of using CEST in routine MRI scanning, utilizing existing hardware in clinics. One recent example of such an approach utilizes machine learning to translate the knowledge from high field CEST to be used at the lower field, resolving spectral ambiguity [37].

In general, the advancement of Artificial Intelligence (AI) algorithms can offer number of exciting possibilities for CEST, from better disambiguation of signals, to better models and shorter acquisition times. Acquisition times pose an additional hurdle for the successful preclinical and clinical applications. Typically, multiple off-resonance and, hence, images are required in order to reconstruct reliable Z-spectrum and provide full information. Advancement of Compressed Sensing (CS) with potential expansion to AI-based algorithms for reconstruction offer a route to

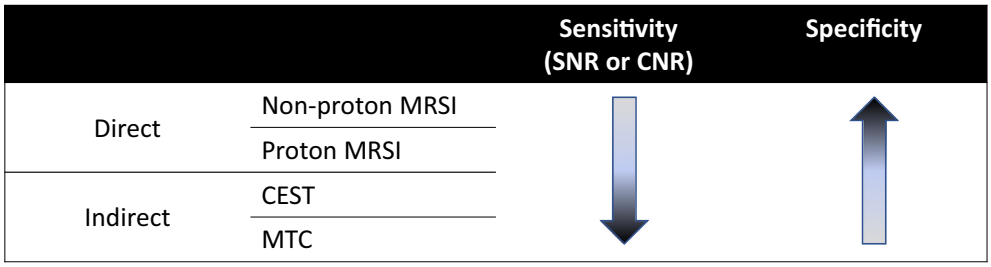

Fig. 1. Simplified schematic of sensitivity and specificity of direct and indirect molecular MR methods discussed in the text.

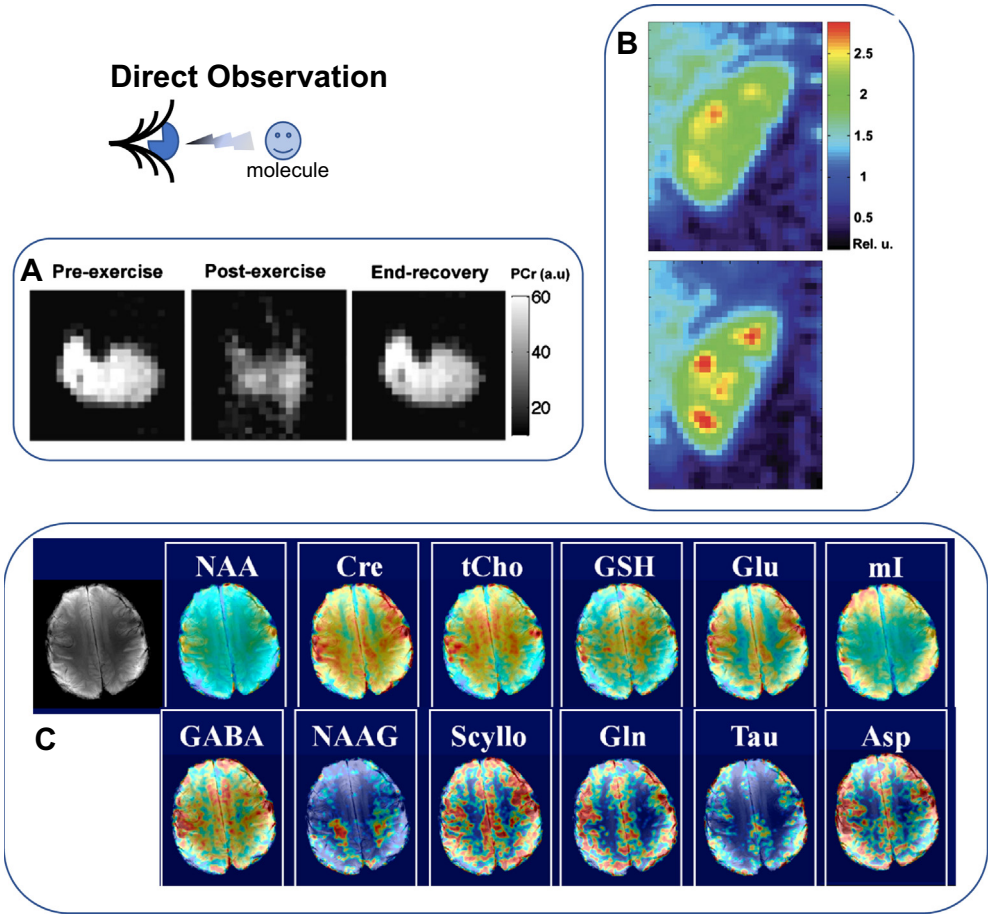

Fig. 2. Examples of "direct observation" of molecular information. (A) Cross-sectional Phosphocreatine map of a human lower leg muscles of before the beginning and at the end of the exercise, as well as at the end of the recovery period at 7 T (Reproduced with permission from Ref. [3]). (B) Central coronal slices of the 3D Sodium images of the human kidney under normal conditions (top) and 12-h water deprivation (bottom). (Reproduced with permission from Ref.[47]). (C) Ultra-high resolution (128 × 128) metabolite maps of human brain for 12 different metabolites along with the anatomical reference scan (top left) obtained using proton MRSI at 9.4 T (Reproduced with permission from Ref.[48]).

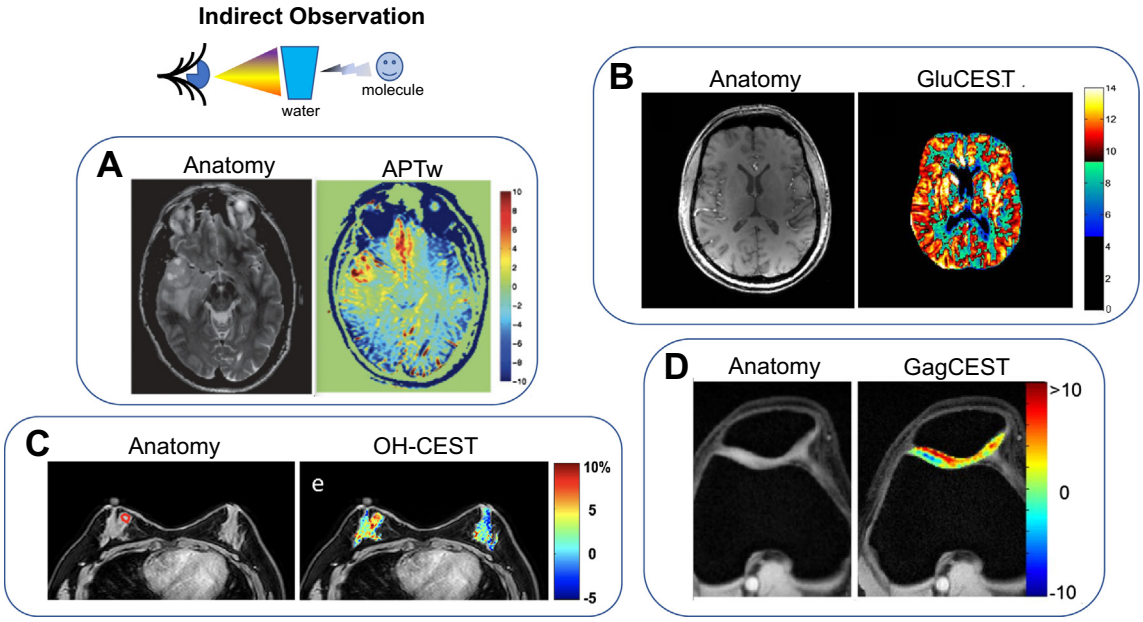

Fig. 3. Examples of "indirect observation" of molecular information using CEST: (A) APT-weighted imaging of a patient with a glioblastoma at 3 T (Reproduced with permission from Ref. [49]). (B) Glutamate mapping in subcortical brain structures (Reproduced with permission from Ref. [31]) using gluCEST at 7 T. (C) Hydroxyl-group weighted CEST map of a patient with ER-negative Invasive Ductal Carcinoma at 3 T (Reproduced with permission from Ref. [45]). (D) Glycosaminoglican mapping using gagCEST at 7 T (Reproduced with permission from Ref. [50]).

speed-up CEST applications [38,39]. As a note of caution, even with these advances, it is unclear if ultimate specificity and precise identification could be ever achieved in realistic acquisition times, taking into account the complexity of the in-vivo processes and how many different levels (molecular, cellular, networks) can be involved before the signal is ultimately detected by MRI.

The discussion of CEST is not complete without mentioning MTC. Recent developments of quantitative MT (qMT) offer an important window into dynamic processes and provide information correlating with myelin content [40,41]. An interesting new method, inhomogenous Magnetization Transfer (ihMT) employs dual saturation to create contrast selectively based on residual dipolar interaction [42]. The approach offers more direct visualization of myelin in brain. This is an exciting new development, since it offers utilization of residual strong dipolar coupling, typically treated as an "enemy" leading to line broadening and generally unobservable signals in MRI. It is tempting to speculate that the future will bring further advancement and dissemination of new (and old) approaches, where a molecular specificity (and sensitivity) is improved via greater utilization of interactions such as residual dipolar coupling or specific relaxation pathways.

Perhaps, the challenge should not be "how to improve molecular MR specificity and sensitivity?", but "can molecular MR be utilized to provide relevant clinical or biological information?". For example, correlation of CEST with the proliferation index Ki-67 that had been observed in brain and breast tumors [43–45], can provide relevant information for patient care, without answering (currently) what molecule exactly caused the MRI signal to change. In such applications, we can envision the use of AI in identifying more and more interesting correlations of CEST or MTC (or Spectroscopy) with pathology or even genetics. One hope is that these will further open a path to radiogenomics: delivering pathological and genetic information from images.

Finally, advancement of AI approaches and large databases offers interesting and exciting possibilities. We can envision a creation of large imaging databases, with contributions from molecular MRI, but also clinical MRI and other imaging modalities (CT, tissue imaging, optical) as well as pathology, genetics and patient outcomes (current example is The Cancer Imaging Archive [46]). Innovative algorithms could search and identify links and correlations between different markers with the ultimate goal of improving our understanding of a disease and/or patient care. These can be achieved via identification and generation of in-vivo imaging protocols that provide meaningful histopathological and even genetic correlates and predictors. The full utilization of the breadth of information and full force of technology (e.g. AI) in such setting would require collaboration from multiple institutions and organizations. There may be multiple obstacles for this approach, including cyber-safety and privacy concerns. Nevertheless, such utilization of all available information may be a reality in not-so-distant future. Indeed, large patient databases containing relevant medical and imaging information are already being created. Tech giants are actively involved in medical datamining. We can expect that molecular MRI applications will become part of a larger data coordination infrastructure.

In summary, molecular imaging using MRI offers exciting possibilities. While challenges associated with sensitivity and specificity remain, we can expect increased utilization of MR methods to study molecular-level events in-vivo and in humans. Paraphrasing a quote: "If you can imagine it (molecule) you can image it".

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