Contra-CEST

Sunrise Debate: Clinical Utility of CEST in Oncology Course

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Target audience: Those with a basic understanding of the method who are considering implementing a CEST imaging protocol from an oncological perspective.

Objective: To acknowledge the negative aspects of CEST imaging and pitfalls that may be encountered as an aid to deciding whether CEST is a worthwhile approach to explore for the diagnosis of cancer and the assessment of therapy.

Introduction

With all the buzz surrounding the topic of chemical exchange saturation transfer (CEST) as a "new", molecule-specific contrast mechanism with promises of pH imaging, it is no surprise that there are many people interested in implementing it. This talk will provide a reality check, discussing the caveats that go along with the expectations, and the many challenges that stand in the way of an ideal CEST protocol.

CEST contrast can be separated into two broad categories: 1. endogenous CEST[1, 2] and CEST from biological molecules [3, 4], and 2. exogenous CEST contrast agents[5, 6]. Endogenous CEST arises from molecules native to the cells: amide protons on the protein backbone, glycosaminoglycans in collagen, etc. These protons resonate between approx. 0-5 ppm of the water resonance. Exogenous CEST contrast agents such PARACEST are typically chelates of paramagnetic ions (often a lanthanide), which can shift the resonance frequency into the hundreds of ppm. Each group shares common concerns related to nomenclature, sensitivity and specificity, protocol development, RF power, and data analysis, each from a slightly different perspective due to differences in concentration, resonance frequency and exchange rate of the labile protons. The exogenous contrast agents have additional issues regarding biocompatibility.

Sensitivity and specificity

The sensitivity of CEST can be defined as CNR/unit concentration (a.k.a. proton transfer enhancement[4]) of the detectable moiety, with further refinements taking scan time into consideration (CNR efficiency[7]). In an ideal case, the bulk water protons could be completely and exclusively saturated through transfer of saturation from the CEST protons[8]. This is physically impossible due to finite relaxation times, resulting in saturation efficiency below 100%, and there are further limitations due to low concentration. PARACEST applications anticipate CEST-specific signal changes of upwards of 50% from phantom studies [5, 9, 10], but this is hardly realized in preclinical applications in which signal changes in tumours do not exceed 5-10%[11, 12]. Endogenous CEST is limited to approximately 5% in tumours[9]. Specificity and sensitivity are hampered by the presence of a multitude of additional factors which affect the contrast, including RF power[13], T1 and T2 relaxation, field inhomogeneities[14], other CEST pools[15, 16], and magnetization transfer from semisolid protons[17]. Several methods have been developed which are less sensitive to instrumental and other non-CEST related parameters[18-21], however the most failsafe method for removal of these terms involves the time-consuming acquisition of parameter maps, requiring the addition of T_1, B_1, B_0 etc. mapping to the protocol[17]. To make matters worse, when employing the most straightforward CEST quantification (CESTR asymm) the endogenous CEST from amide protons which resonate at 3.5 ppm is influenced by the presence of NOE

from aliphatic protons which resonate at ~-3.5 ppm[22] which is also increased in tumours. Endogenous CEST is specific to a type of labile group (amide, amine, hydroxyl, etc) however there are many subclassifications so that all the endogenous CEST spectra are composed of groups of compound peaks[23], limiting the ultimate specificity of the technique. Since labile proton relative concentration or exchange rate are correlated in the CEST model[25, 26], it may be impossible to discern which property is causing the observed contrast changes, especially when both protein concentration and pH are expected to change within the tumour. Furthermore, with endogenous CEST, the low CNR severely limits the accuracy and precision of fitting to quantitative models. The majority of CEST experiments involve the generation of "negative contrast", in that the saturated signal is less than the reference signal. This poses a problem for CEST interpretation, and post-processing must occur so that regions of high CEST "light up" in an image instead of appearing darker. It also has the consequence that images with more CEST will have a lower SNR due to the reduced signal.

RF power

In order to obtain the maximum saturation efficiency[27, 28], it may require a saturation pulse with an RF amplitude which exceeds the safety limits [29-31]. RF amplitudes ranging from 1 – 20 μT have been used in preclinical studies of CEST in cancer. Even if a strong RF pulse was permissible, spillover effects will cause the observed CEST effect to be reduced by direct saturation of the water resonance[32]. There may not be an acceptable compromise between RF power limitations and minimum acceptable CNR. Increasing the field strength will increase the separation (in Hz) between CEST peaks and the water resonance, and provide increased SNR, however the deposited RF energy increases approximately with the square of the RF amplitude causing further restrictions on the CEST experiment.

Protocol and Analysis

As of this writing, CEST protocols are not available by default with any of the vendor's standard pulse sequence packages, and consequently a considerable amount of expertise is required to set it up at a new location. Often, CEST pulse sequence development involves overcoming limitations on maximum pulse length, and before the protocol becomes reasonably "user friendly" scripts or automation must be implemented so that the manifold images required for a CEST spectrum can be grouped and the offset frequencies easily assigned. Protocols optimized for 3 T [27, 33, 34] must be completely revised for 7 T[35] in light of the tighter restrictions on RF power. Since most vendors specify RF frequency in terms of Hz, rather than ppm, offset frequencies must also be recalculated when the field strength changes.

Despite this fact, numerous institutions have implemented the CEST sequence, and preclinical[15, 36-39] and clinical trials[27, 40-46] are underway. As CEST applications are in their infancy, with each new implementation improvements are made to the protocol and as a result there is no standard method. Optimizable parameters include the RF pulse strength and shape, TR, and choice of offset frequencies [10, 47, 48]. This limits the ability to compare CEST results between studies, especially in cases where the metric is "CEST contrast" (i.e. CESTR asymm[49] or peak amplitude[15, 21]) rather than a fundamental quantifiable property such as CEST pool concentration or exchange rate constants[50, 51]. Efforts have been made to reduce the scan time for CEST protocols[34, 52-54], but they largely remain time consuming and difficult to fit into a reasonable (i.e. 20 minutes) scan period, much less have sufficient temporal resolution[55] to capture immediate dynamic changes due to treatment.

Depending upon the completeness of the acquired CEST spectrum data, there are several options for correction of artifacts and inhomogeneities[14, 56-58], and a choice of CEST metrics to be determined. Full parameter quantification, however, of a multi-compartment Bloch equation model is impractical (difficult to implement, time consuming to run fitting). Parameters may be fixed to save processing time,

however deciding on what parameters to fix at what value and the impact of this choice upon the unfixed parameters is not immediately clear.

Nomenclature

CEST Z-Spectra are preferentially displayed in order of decreasing offset frequency from left to right. This has its origins in NMR spectroscopy, where historically the axis was ordered from low shielding to high and the offset frequency is highest for the lowest amount of shielding. Ordering the x-axis from positive to negative values is the source of much confusion for those approaching CEST from a non-spectroscopic background. Worse, if one was to mistakenly switch the axis orientation, the presence of the aliphatic NOE at the incorrectly assumed position of amide CEST is misleading to the extent that the error may not be immediately realized. Even for those who are familiar with the axis convention, the reference point of the ppm axis is changed for CEST so that instead of a reference chemical (such as TMS) being denoted as 0, this is now shifted so that water protons are at 0 ppm.

There are many different quantitative and semi-quantitative methods of expressing the CEST metric, and one must take care to recognize the differences between CESTR asymm[32], relative CEST pool size[59], and CEST peak amplitude[60] (to list a few). There are two schools of thought for expression of the exchange rate, as forward and backwards rates k_{sw}/k_{ws} [59] or to wrap these into a single pseudo-first order rate constant, R[61]. There are multitudes of (not necessarily mutually exclusive) applications with the naming convention "__CEST", referring to the labile exchange group or another distinguishing quality of the CEST contrast agent [62-65]. These imaging applications all follow similar protocols, but have their own set of optimized scanner parameters.

Biology and Chemistry

CEST protocol development and experimentation requires a solid background in biochemistry (and chemical synthesis for the contrast agents), in addition to MRI physics. Knowledge of labile species, range of possible exchange rates and how these vary with solvent accessibility and pH are a few of the major issues. CEST contrast agents must be engineered to have exchange rates within an optimal window and with the offset frequency as large as possible[50, 66]. It is difficult to replicate the biological conditions and thus CEST contrast agents tested in phantoms often have markedly better performance than in vivo where unpredictable bonding may occur[67] and there is the presence of competing magnetization transfer pathways[9].

In order to interpret CEST images of cancer, one must be aware of which biological compartment is measured by the CEST experiment – mainly intracellular for endogenous amide CEST due to the higher concentration of proteins and peptides[2], and extracellular for PARACEST due to the accumulation of extravasated contrast without uptake by the cells[68-70]. Intracellullar and extracellular pH behave differently depending on the pathology or treatment [71-75], and the sensitivity of the CEST experiment to changes in these compartments must be determined prior to embarking on large scale studies. Changes in pH (especially intracellular) are small due to the mechanisms for maintenance of homeostasis[76].

CEST contrast agents

An immediate concern with CEST contrast agents (namely PARACEST), as with any injected compound, is toxicity to humans and the associated lengthy approval process for those deemed safe in pilot preclinical trials. PARACEST agents are similar in structure to Gd-containing counterparts used as T1-shortening agents; the chemistry is also similar and the stability of the compounds is more closely related to the ligand/chelate than the lanthanide ion[67]. Paramagnetic ions are coated, chelated or

incorporated into macromolecular structures during preparation, which all but eliminates their toxicity[77]. However, PARACEST agents larger than 5nm evade rapid clearance by the kidneys[78] and high concentrations can accumulate in the tissue once leaving the blood pool[79]. Over time, if the agents remain in the system (often in patients with impaired kidney function) the agents can break down to reveal the toxic core.

Oncological applications of CEST and alternatives

CEST has been proposed as an MRI contrast mechanism sensitive to protein concentration and pH, however the question remains whether it is more effective than pre-existing imaging methods with similar objectives. Although CEST performs well at identifying regions of tumour, with the exception of recurrent cancer in the presence of radiation necrosis[80], it does not appear that CEST offers improved diagnostic potential over standard methods (i.e. T1- and T2- weighted imaging, DCE-MRI). There is potential in CEST for identification of hypoxic regions which have increased glycolysis[81] and may be resistant to treatment, but it is unclear whether this offers sufficient advantages over FDG-PET. Endogenous pH imaging is an enticing target for CEST experiments, however absolute pH imaging with CEST remains elusive[82, 83]. CEST images which are simply "pH-sensitive" may not be accurate enough to be helpful for evaluation of pH-triggered drugs[84-86].

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