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GlucoCEST Imaging with On-Resonance Variable Delay Multiple Pulse (onVDMP) MRI

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Abstract

Purpose—To examine the detection sensitivity for the rapidly exchanging hydroxyl protons of D-glucose using the recently developed on-resonance variable delay multi-pulse (onVDMP) chemical exchange saturation transfer (CEST) technique.

Methods—The onVDMP method was applied for the detection of water signal changes upon venous D-glucose infusion in mice with 9L glioma xenografts. The effect size of onVDMP MRI during infusion was compared with that of conventional continuous wave (CW) CEST MRI.

Results—Both methods highlighted the tumor and the blood vessels upon D-glucose infusion. In interleaved studies, the mean signal changes detected by onVDMP were found to be 1.8 times higher than those by CW-CEST, attributed to its high labeling efficiency for fast exchanging protons and the labeling of the OH protons over a larger frequency range.

Conclusions—The onVDMP method is a more sensitive technique for the detection of exogenous CEST agents with fast-exchanging protons compared to CW-CEST MRI.

Introduction

D-glucose can be used as a natural biodegradable contrast agent for MRI by exploiting the chemical exchange properties of hydroxyl protons (1–3). Several MRI methods have been applied to monitor glucose uptake and transport *in vivo* in animals, including conventional

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continuous wave chemical exchange saturation transfer (CW-CEST) (1,2,4,5), R_2 relaxivity enhancement (6,7) and chemical exchange sensitive spin locking (CESL) (8–10). Since intravenous injection of D-glucose is approved for glucose tolerance testing, translating these glucose detection techniques to human studies has been rapid, successfully showing application to the detection of D-glucose in brain cancer (11–13). When D-glucose enters the blood stream, it is actively taken up into the brain by facilitated transport via glucose transporters at the blood brain barrier (BBB) (14,15) and subsequently transported into the cells and metabolized. When there is a leakage in the BBB, glucose can also enter the extravascular extracellular space (EES) through diffusion, in a way similar to conventional gadolinium based contrast agents. Therefore, glucose can be a suitable alternative contrast agent for brain tumor detection. Due to angiogenesis (higher blood volume) and disruption of the BBB with leakage into extracellular space, tumors are enhanced in glucoCEST compared to normal tissues (4).

Gd based contrast agents have been widely used to assess perfusion and permeability (16). In comparison, D-glucose may be more sensitive to detect early alternations in the vascular permeability caused by tumors due to its smaller size. The polar surface areas (PSAs) of Gd based contrast agents currently approved in the US to image BBB degradation range from $440 \text{ \AA}^2$ (MultiHance, Gadobenic acid) to $210 \text{ \AA}^2$ (Magnevist, Gadopentetic acid). As a consequence, the BBB needs to be disrupted significantly to allow Gd to leak in. In comparison, D-glucose has a PSA of $110 \text{ \AA}^2$ and can diffuse into the lesions much easier; therefore, it is expected to be more sensitive to minor BBB disruptions, and possibly to detect lesions at an earlier stage than Gd. Previously we have demonstrated the feasibility of detecting brain tumors using dynamic glucose enhanced (DGE) imaging with CW and pulsed CEST MRI methods (4,11). The high temporal resolution (10 s) was achieved by applying the saturation RF pulses at a single frequency, i.e., 1.2 ppm. However, each glucose molecule contains five hydroxyl protons (1.2, 2.2 and 2.8 ppm) that exchange with bulk water protons at exchange rates (k_{sw}) between about 3 to 6 kHz at physiological pH and temperature. In order to label most of these fast exchanging protons efficiently using conventional CW-CEST, high power RF pulses have to be applied, which limits the feasibility of glucoCEST studies on clinical scanners. Also, the saturation power is limited by the saturation offset of OH groups being to water resonance, because, at high saturation power, the direct saturation of water signal will obscure CEST signals of protons close to the water resonance. To make matters worse, fast exchange leads to coalesced signals in the 0–3.5 ppm range in the Z-spectrum and the maximum signal intensity need not be at the particular OH resonance frequencies (17–20). In this study, we employ the recently developed on-resonance variable delay multi-pulse (onVDMP) method to detect the glucose uptake upon infusion (21). A sensitivity gain by onVDMP is expected to be achieved because of an increased labeling efficiency due to the application of a strong RF field B_1 , which excites exchangeable protons over a broader frequency range. We first used Bloch simulation to optimize the length, strength and delay between labeling pulses to make onVDMP sequence sensitive to the fast exchanging hydroxyl protons in D-glucose. We then applied the method on a mouse glioma model and compared the glucoCEST signal obtained by onVDMP and standard CW-CEST on the same animals using an interleaved scanning approach. The results show that a higher glucoCEST signal can be obtained using the

onVDMP method, demonstrating its potential for CEST MRI of glucose and its derivatives (22–24).

Methods

On-resonance Variable Delay Multi-pulse (onVDMP) Sequence Figures 1A&B illustrate the onVDMP and CW-CEST preparation pulse sequences, each followed by the same imaging readout. The onVDMP sequence consisted of a train of 32 binomial RF pulses with the offset on the water resonance and a 10 ms mixing time (t_{mix}) between pulse pairs. The binomial pulses consist of two block pulses of opposite phase. The width of these saturation pulses was 1.5 ms (3 ms for the binomial pair) with a peak power of 15.6 μT , corresponding to a flip angle of 360 degrees and 1.73 J of RF energy deposition. The flip angle was chosen to minimize image artifacts due to possible residual transverse magnetization. The excitation profile of the binomial pulses of different length was simulated and experimentally tested using an ultra-fast gradient encoding CEST method (25,26) on a water phantom with a single pair of binomial pulses. For CW-CEST, a single magnetization transfer (MT) pre-pulse of 2s duration and a B_1 of 1.6 μT (corresponding to 1.52 J of energy deposition) was used at an offset of 1.2 ppm.

The normalized change of water magnetization, $M_{\text{water}}/M_{\text{control}}$, was simulated for both sequences using the Bloch equations by assuming 4 pools, i.e. a water pool and three exchanging proton pools. The water magnetization with no exchangeable protons present is denoted as M_{control} . The simulations were performed both at 11.7T and 3T.

MRI acquisition was done using a Rapid Acquisition with Relaxation Enhancement (RARE) sequence, $\text{TR}/\text{TE}=5.0 \text{ s}/3.8 \text{ ms}$. With a RARE factor of 23 and partial Fourier acceleration factor of 1.4, each image was acquired with two segments resulting in a dynamic scan time of 10 s. A single slice with $0.25 \times 0.25 \text{ mm}^2$ in-plane resolution and 1 mm thickness was imaged within a FOV of 1.6 cm^2 .

Phantom

Phantoms with just PBS and 25mM D-glucose in PBS were prepared, with the pH adjusted to 7.2. The phantoms were imaged at 37°C using the same parameters as for the animal experiments.

Animal preparation

The study was carried out under the approval of the institutional animal care and use committee. 9L glioma cells were implanted (0.05×10^5 cells/ μl) by stereotaxic injection into the right caudate/putamen of 8 female severe combined immune deficiency (SCID) mice. Imaging experiments were performed between day 8 and 11 post implantation. Animals were not fasted overnight prior to experiments and had access to water on the day of experiments. The tail vein was cannulated with a catheter for glucose infusion before the MRI scans.

Imaging Experiments

An 11.7T horizontal bore Bruker Biospec scanner with a 23 mm volume transceiver coil was used. Animals were anesthetized using 2 to 2.5% vaporized inhaled isoflurane, followed by 1 to 1.5% isoflurane during the MRI scan. Anatomical images were acquired to identify the slice with maximum tumor size. Two groups of mice were studied. In group one (Group I, n=4), the onVDMP and the CW-CEST methods were acquired in an interleaved fashion post the glucose injection, which was aiming to properly compare the sensitivity in measuring glucose uptake on the same animals. Prior to the injection experiment, field maps were acquired and first and second order shimming performed over the mouse brain. The typical water linewidth for the shimmed volume was around 50Hz. S_0 images prior to injection were collected by setting the saturation offset at 200 ppm with the same CW-CEST sequence. Six onVDMP, CW-CEST and S_0 images each were collected as baseline, after which a bolus of 0.15 mL 50% w/w glucose (0.5 g/mL, clinical-grade Dextrose, Hospira) was given over 60 s through the tail vein using a syringe pump (Harvard Apparatus). When the injection was completed, the MRI acquisition with alternating CW-CEST and onVDMP imaging blocks was started. Each CW-CEST or OnVDMP acquisition block consisted of six continuous CW-CEST or onVDMP image acquisitions with a total scan time of 73 s. The acquisition scheme was designed to ensure the water signal reaches steady-state for the CW-CEST/onVDMP experiments. Furthermore, the images in each block can be averaged together to enhance the SNR. Both sequences were performed using the parameters described in the simulations.

In order to monitor the dynamic change upon glucose injection with high temporal resolution (10 s for each image), another group of mice (Group II, n=4) were imaged pre, during and post glucose injection. The dynamic scanning started before glucose injection by acquiring 18 baseline scans over three minutes. Without stopping the scanning, a bolus of glucose was injected with the same protocol described above. The dynamic acquisition lasted one hour following injection.

Spectroscopy

To investigate the source of the signal after infusion, localized ^1H magnetic resonance spectroscopy (MRS) was acquired using a stimulated echo acquisition mode (STEAM) technique with outer volume suppression (OVS). The water signal was suppressed by the variable power RF pulses and optimized relaxation delays (VAPOR) method with 300 Hz bandwidth (27). MRS spectra were recorded prior and one-hour post glucose injection, respectively. Localized shimming up to second order was performed using field mapping before each MRS acquisition. The sequence parameters were $\text{TR} = 2500$ ms, $\text{TE} = 3$ ms, mixing time $\text{TM} = 10$ ms, number of acquired complex points = 2048, $\text{NEX} = 256$ and a total acquisition time of 10 min 40 s. A single voxel of $1.8 \times 2.2 \times 2.0 \text{ mm}^3$ was chosen encompassing the tumor region.

Data analysis

All CW-CEST and onVDMP images were normalized to the pre-injection averaged S_0 image. Dynamic difference images (with signal intensity S) were generated by taking the difference between each dynamic image and the average of all pre-injection images:

$$\Delta S(t_n) = \frac{\bar{S}_{base} - S(t_n)}{\bar{S}_0} \quad [1]$$

Since no magnetization transfer ratio asymmetry was calculated, no B_0 or B_1 corrections were necessary for the broad hydroxyl signal.

The area-under-curve (AUC) image, which reflects contrast agent uptake and retention, was calculated by summing n images within certain time period:

$$AUC = \sum_1^n \Delta S(t_n) \quad [2]$$

For the comparison study (Group I), five image blocks were summed with each block having six images, which produced difference images representing 12 min of total scan time after glucose injection. Average signal intensities over all voxels in the tumor and a contralateral ROI of similar size were compared.

The signal to noise ratio (CNR) was defined as the difference in signal intensity between the tumor S_{tumor} and contralateral brain S_{contr} , divided by the standard deviation of the contralateral brain region, which reflects measurement noise.

$$CNR = \frac{S_{tumor} - S_{contr}}{sd(S_{contr})} \quad [3]$$

Results

Figure 1C and 1D shows the simulated water signal variation as a function of exchange rate for the CW and onVDMP saturation at 11.7 and 3 Tesla, respectively. It can be seen that for slower exchange rates (<1.5 kHz), the CW-CEST method is more sensitive compared to onVDMP. Meanwhile, the sensitivity of CW-CEST decreases for protons with higher exchange rates while the sensitivity of onVDMP first increases and then levels off but stays higher than CW-CEST. At 11.7 T, when using binomial pulses with different bandwidths, the onVDMP signal always exceeded CW-CEST signal at higher exchange rate. Using a 4 ms binomial pulses yielded the highest signal for exchange rates larger than 1.5 kHz. However, when using 4 ms or longer binomial pulses experimentally, black-band artifacts resulting from B_0 and B_1 variation were observed due to the much narrower excitation bands created by the longer pulse. Therefore 3 ms binomial pulses were chosen in experiments, which gave higher signal for rates above 2 kHz compared to CW-CEST. At 3T, the length of the binomial pulses was adjusted to 12 ms to achieve a similar excitation bandwidth as at 11.7T. The simulation shows that the onVDMP method leads to a higher signal at 3T at all exchange rate.

The saturation/excitation profile as a function of offset by one binomial pulse is shown in Figure 2A. It can be seen the labeling efficiency with respect to offset is a periodical function when using an excitation pulse of different bandwidth, while the water magnetization is attenuated slightly due to T_2 relaxation. The simulation demonstrates that a pulse width of 3 ms has an excitation profile between 0.5–2 ppm, which is ideal for labeling most of the exchanging hydroxyl protons of glucose. In the experimental profile measurements, the method does not distinguish +Mz and –Mz since only the magnitude of the signal is being detected. The experimental results shown in Figures 2B and 2C agree well with the simulation.

The signal difference between the PBS and 25mM Glucose in PBS phantoms is defined as glucose contrast ($M/M_{control}$). For onVDMP and CW-CEST sequences, the contrast are 13.8% and 9.4%, respectively.

The dynamic difference images acquired using onVDMP and CW-CEST sequences from a representative mouse are shown in Figures 3A and 3B. For the low time resolution used (73s), the tumor, blood vessel and ventricle contrasts emerge right after the glucose infusion. The contrast persists during the whole course of the imaging experiment. The dynamic curves show that the glucose signal difference (Figure 4A) acquired by the onVDMP method is bigger than that by CW-CEST. The same is the case in the AUC_{12min} images (Figure 4B). When averaging the AUC_{12min} signal in the tumor region for the mice in Group I (n=4, Figure 5A), the onVDMP method yields an average signal change of $18.3\% \pm 5.8\%$, which is significantly higher than the CW-CEST signal change of $10.0\% \pm 3.4\%$ ($p=0.011$). In terms of CNR, the onVDMP AUC_{12min} image (2.72 ± 1.0) is also significantly higher than the CW-CEST AUC_{12min} image (0.82 ± 0.3) with $p = 0.027$ as shown in Figure 5B.

The dynamic glucoCEST signal changes by onVDMP for the mice in Group II (n=4) are presented in Figure 6, for both tumor and contralateral brain region. It is evident that the tumor signal change is much greater (7% – 15%) than the contralateral brain change (2% – 4%). The results also show that the dynamic curves vary significantly among mice in the tumor, which we attribute to the heterogeneity of the tumor, i.e. more or less angiogenesis and different size of the extravascular extracellular space, or possibly some partial volume effects with normal tissue and vessels. Therefore, the individual dynamic curves are presented instead of a mean curve over all mice with a standard deviation. The glucose signal enhancement in contralateral brain is quite uniform and reproducible, supporting our interpretation that the disparity in signal in the tumor region is due to the heterogeneity.

In order to assess the contribution of any increase in the concentration of metabolites with rapidly exchanging protons that may also contribute to the onVDMP signal (e.g. glutamate in normal brain and lactate in the tumor), the metabolic change in the tumor before and after glucose infusion (time point at 1 hour) was examined by in vivo MRS performed over a voxel covering the whole tumor region. The aliphatic glucose resonance peaks in the chemical shift range of 3.0 to 4.0 ppm increased substantially, and are clearly evident in the difference spectrum shown in Figure 7. Interestingly, the products from glucose metabolism, such as glutamate/glutamine (2.1, 2.35–2.45 ppm)(28), and lactate (1.3 ppm) did not show noticeable change over the observed period.

Discussion

In phantoms the onVDMP signal is 1.47 times higher than CW-CEST at 37°C which is consistent with our simulations. Our in vivo results show higher detection sensitivity for the onVDMP preparation compared to the CW-CEST preparation. The onVDMP sequence was originally designed to separate fast magnetization transfer effects in the Z-spectrum, such as from rapidly exchanging amine and hydroxyl protons, from slow-transfer effects, such as from amide protons and relayed Nuclear Overhauser enhancements (rNOEs) from mobile and semi-solid protons. This can be achieved by varying the mixing times while fixing the number of saturation pulses. However, when the number of pulses and a mixing time are fixed, the sequence can be used to monitor the change in CEST contrast agent concentration. Varying the mixing time is not necessary because separation from background signals such as the conventional magnetization transfer contrast (MTC) pool is already achieved by dynamic acquisition of signals during infusion of the CEST agent and processing of difference data relative to before infusion. If a large number of saturation/excitation pulses with relatively high power are applied, such as in the current experiments, the on-resonance binomial saturation pulses in the onVDMP can achieve a high labeling efficiency for fast-exchanging protons. The allowed power level for conventional CW- and pulsed-CEST techniques is more limited on clinical scanners. On the other hand, the direct water signal saturation in onVDMP is mainly determined by the length of the pulse, and is not sensitive to the pulse power. This is in contrary to the CW-CEST sequence. When a train of binomial pulses is applied on the resonance with water offset, water proton magnetization is maintained along the z-axis by the jump-and-return type binomial pulses. For protons resonating at other frequencies, the chemical shift evolution of transverse magnetization that occurs during the pulses can not refocused. As a consequence, the resulting longitudinal magnetization of the solute protons is attenuated after each binomial pulse pair in a manner depending on the chemical shift from water resonance. Thus the excitation profile of the binomial pulse is a periodical function and covers a few bands of chemical shifts. The location of the bands is determined by the pulse width (Figure 2), while the full range of coverage, i.e. the number of bands, is determined by the peak power of the pulses. For the power applied in current study, the envelope is close to 5 ppm (Figure 2). While there are maxima and minima, an appreciable fraction of the five hydroxyl protons in each glucose molecule are affected. On the contrary, the dynamic CW-CEST images are acquired only at 1.2 ppm, where just three out of the five hydroxyl protons (29) contribute. Together these factors (exchange rate sensitivity and bandwidth) result in a higher signal obtained by the onVDMP sequence compared to CW-CEST.

Another advantage of the onVDMP method is the much shorter total labeling time (0.406 s) comparing to the CW-CEST (2 s). Although the TR was set to 5 s in both experiments to keep the dynamic scan time the same, the TR could be shortened further in onVDMP to achieve a higher temporal resolution and/or temporal SNR compared to CW-CEST.

Brain tumors can grow rapidly on a day-to-day basis and are highly heterogeneous, which is evident from the dynamic studies in Figure 6A. Therefore interleaved acquisition of onVDMP and CW-CEST methods was needed as a fair means of comparing the sensitivity of the two methods. One possible caveat is that the first time points acquired by CW-CEST

(at 75 s) and onVDMP (150 s) post injection may reflect differences in glucose concentration rather than the detection sensitivity due to the strong glucose gradient between blood and tissue. However, the later time points reflect the difference in acquisition sensitivity (Figure. 4A).

glucoCEST signal detected by onVDMP

Since onVDMP is favorable for detecting fast-exchanging protons, any change in concentration of any metabolites during the glucose infusion would also contribute to the onVDMP signal. It has been well investigated by ^{13}C -glucose infusion of normal brain that the metabolic products from glucose usually emerge about 10–20 minutes after injection when D-glucose has entered the tricarboxylic acid cycle (30–33). Among the metabolic products, a change of glutamate concentration would be the major confounder in the observed signal due to the broad amine resonance coalesced with water (19,20). For tumors, ^{13}C -glucose studies have shown a rapid rise in lactate, reaching a maximum within 10 min (34,35), which may also contribute to the CEST signal (36,37). Normally, the concentration of glutamate is ~8–10 mM in the brain, ~0.5–2 μM in the extracellular fluid and 50–100 μM in the plasma (38). Also it has been reported that the blood glutamate decreases upon glucose infusion (39). Therefore we conducted a MRS study to investigate if the change in glutamate concentration plays a role in the observed signal. The comparison between the MRS spectra in a tumor region acquired before and after the glucose infusion showed that the concentration variation of glutamate and other major metabolites was not noticeable, while only the glucose level increased significantly. This is in line with glutamate data from ^{13}C studies (40), which show a change in ^{13}C glutamate, but not total glutamate. This observation confirms that the majority of the signal changes detected by the onVDMP method originated from glucose itself.

Brain tumor cells have high metabolic demands as they go through increased cell division. To keep up with the demand, angiogenesis occurs within the tumor. The newly formed blood vessels tend to be leaky and the ratio of extracellular extravascular space to cells increases in the tumor. Together, the increased vascularity and leakiness into extravascular extracellular space are responsible for the increased DGE contrast in the tumor compared to the contralateral brain. Comparing the dynamic signal curves between tumor and brain, it is evident that in the contralateral brain where the BBB is intact, the signal mainly originates from the vascular compartment and intact normal brain tissue (41), which has a concentration that is much lower (~1/4 of vascular). As such, the DGE curves are quite consistent over the 4 mice that were studied. On the other hand, the signals from the tumor regions are mainly from vascular and increased EES contributions. After the initial signal increase immediately following glucose infusion, the signal change exhibits a rather scattered pattern between different mice, which we attribute to the heterogeneity of the brain tumors and some partial volume effects of large vessels.

Clinical Translation Potential

The main difference between 11.7 T and 3 T is in the frequency offsets (in Hz) of the hydroxyl protons of glucose. At 3 T, there is a situation of fast to intermediate exchange on the MR time scale because the exchange rates of the hydroxyl protons (>3000 Hz) are on the

order of magnitude of their radial frequency offsets with respect to water (942 – 1,885 rad/s). Then, the peaks of the hydroxyl protons are partially coalesced with the water peak. Due to the much smaller offsets of the hydroxyl protons at 3 T, the saturation pulses applied in CW-CEST to label glucose will inevitably induce strong water direct saturation, which partially obscures the CEST contrast. With onVDMP, on the contrary, higher power can be used since the water resonance is barely attenuated. However, the pulse width of the binomial labeling pulses in onVDMP need to be much longer for 3T to label the hydroxyl protons. When using 32 binomial pulses with a pulse width of 12 ms, both the onVDMP and CW-CEST signals decrease for exchange rates higher than 500Hz, yet the onVDMP method leads to a higher signal than CW-CEST for all exchange rates according to our simulations. At clinical field strength, the duration of the pulses and the mixing time between binomial pulses can be further tuned to achieve high levels of saturation of the fast exchanging protons while minimizing water direct saturation, which makes the onVDMP technique suitable for translation to clinical scanners.

CW-CEST method usually requires long saturation on the order of one to two seconds and, although the specific absorption rate (SAR) is usually not a main issue, the implementation on the clinical scanners is often difficult due to the amplifier limitations. Currently, most CEST studies on clinical scanners are performed with a train of saturation pulses interleaved with amplifier settling delays due to the amplifier duty cycle limitation. Such an approach decreases the saturation efficiency of fast-exchanging protons significantly (42) as they require high B_1 to achieve sufficient saturation. Using the simulation parameter above, we can calculate the SAR to be 54% and 68% for the CW-CEST and the onVDMP sequences respectively assuming same turbo spin echo readout and $TR = 5$ s. While onVDMP has slightly higher SAR than the CW-CEST, it is still well within the limitation making it feasible for clinical translation.

Conclusion

We demonstrate that the recently developed on-resonance variable delay multi-pulse method can be used to study glucoCEST signal changes upon venous glucose injection. Compared with the continuous wave CEST method, the onVDMP method provided higher detection sensitivity due to the excitation of a larger frequency range with signal from fast exchanging protons. Even though the onVDMP method is not very frequency selective, it has the potential for widespread application for dynamic monitoring CEST contrast agent and drug delivery for compounds with fast-exchanging protons.

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Figure 1.

Illustration of the on resonance VDMP (A) and the continuous wave CEST (B) sequences. (C) Simulated CEST signal intensity at 11.7T, i.e. water magnetization reduction, as a function of exchange rate for CW-CEST at 1.2 ppm and onVDMP. $M_{control}$ is the magnetization without exchanging protons. In the simulation, a four-pool model is assumed to consist of a water pool ($T_1=2$ s, $T_2=40$ ms) and three solute pools ($T_1 = 1$ s, $T_2 = 30$ ms) with exchanging protons at 1.2 ppm (30 mM), 2.2 ppm (10 mM) and 2.8 ppm (10 mM). For onVDMP method, 32 binomial pulses of 2, 3, and 4 ms with peak power of $15.6 \mu T$ were used in the simulation. The mixing time between each binomial pair was 10 ms. For CW-CEST, a single saturation pulse of 2 s with B_1 of $1.6 \mu T$ was applied at 1.2 ppm. (D) Simulated signal intensity at 3T. For onVDMP, 32 binomial pulses of 12 ms were used, for CW-CEST, same parameters as in (C) were used. The T_1 of water was set to 1.5 s and T_2 to 200 ms.

Figure 2.

(A) Simulated OnVDMP excitation profile for a single pair of binomial pulses with a total pulse length of 2, 3, and 4 ms. The measured excitation profile for (B) 3 ms and (C) 2ms binomial pulses on water determined by applying a gradient and the binomial pulse simultaneously.

Figure 3.

The post glucose infusion dynamic difference images from one mouse acquired by CW-CEST (A) and onVDMP (B). The first image of CW-CEST was acquired 73 s post infusion, and 146 s for the first onVDMP image. Then the acquisition alternated between the two methods at a 146 s interval. The anatomical images are shown in gray scale at the beginning of the series.

Figure 4.

(A) The water signal change detected by onVDMP and CW-CEST in the tumor region as a function of post infusion time. (B) The AUC images of onVDMP and CW-CEST over a period of 12 min post glucose infusion from the same mouse.

Figure 5. (A) Averaged AUC_{12min} signal in the tumor region and; (B) the contrast to noise ratio of the AUC images for the mice in Group I using the onVDMP and CW-CEST methods.

Figure 6. Dynamic signal change as a function of glucose infusion time acquired by onVDMP in the tumor region (A) and the contralateral brain region (B) for the mice in Group II (n = 4).

Figure 7.

in vivo MRS spectra on a typical mouse brain over a voxel containing the tumor prior (blue), post (red) glucose infusion and their difference spectrum (green). Labeled on the spectra: the various protons (H2-H4) of glucose between 3–4 ppm, the protons attached to the C4 carbons of glutamate/glutamine (glx) between 2.25–2.35 ppm, the protons attached to C3 carbon of glx at 2.1 ppm, and the protons of lactate (Lac) and lipid resonance at 1.3 ppm. Notice that these frequencies can be compared with the CEST frequencies by subtracting the water proton offset (about 4.7 ppm).