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CEST MRI of 3-O-Methyl-D-Glucose uptake and accumulation in brain tumors

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Abstract

Purpose: 3-O-methyl-D-glucose (3-OMG) is a non-metabolizable structural analog of glucose that offers potential to be used as a CEST-contrast agent for tumor detection. Here, we explore it for CEST-detection of malignant brain tumors and compare it with D-glucose.

Methods: Glioma xenografts of a U87-MG cell line were implanted in five mice. Dynamic 3-OMG weighted images were collected using CEST-MRI at 11.7 T at a single offset of 1.2 ppm, showing the effect of accumulation of the contrast agent in the tumor, following an intravenous injection of 3-OMG (3g/kg).

Results: Tumor regions showed higher enhancement as compared to contralateral brain. The CEST contrast enhancement in the tumor region ranged from 2.5–5.0 %, while it was 1.5–3.5 % in contralateral brain. Previous D-glucose studies of the same tumor model showed an enhancement of 1.5–3.0 % and 0.5–1.5 % in tumor and contralateral brain respectively. The signal gradually stabilized to a value that persisted for the length of the scan.

Conclusions: 3-OMG shows a CEST contrast enhancement that is about twice as much as that of D-glucose for a similar tumor line. In view of its suggested low toxicity and transport properties across the BBB, 3-OMG provides an option to be used as a non-metallic contrast agent for evaluating brain tumors.

Keywords

3-O-Methyl Glucose; CEST imaging; contrast enhancement; brain tumor

INTRODUCTION

Natural D-glucose is currently being investigated as a biodegradable alternative for contrast-enhanced MRI studies for tumor diagnosis using Chemical Exchange Saturation Transfer (CEST) MRI (1–3) as well as $T_1\rho$ -relaxation based approaches, also called Chemical Exchange Spin-Lock (CESL) MRI (4–7), and also being translated to the clinic (4,8). D-glucose, being the prime source of energy in the tumor, however, gets rapidly metabolized through glycolysis (9). This leads to a decrease in the CEST weighted signal. Glucose analogs that don't get metabolized are also being explored as alternatives. For instance, 2-deoxy D-glucose (2DG) gets phosphorylated by hexokinase but doesn't get further metabolized (10). The uptake of 2DG has shown to be detectable through CEST and CESL MRI in in-vivo animal studies (11–14) and its fluoro derivative, 2-fluoro-deoxy-D-glucose (^{18}F -2DG), is routinely used in PET/MRI (15–18). However, the toxicity of these deoxy-glucose analogs at the dosage required for CEST-MRI limits hope for human translation (19).

3-O-methyl glucose (3-OMG) is another structural analog of glucose with 4-hydroxyl moieties. It was used in the 80s-90s to study the glucose transport across the blood brain barrier (BBB) as it has no metabolic by-products (20). It has been found that the transport of 3-OMG is facilitated through the glucose transporters (GLUT-1 and 3) and competes with glucose for access to these transporters in the brain (21). There have not been any reported toxicity studies on 3-OMG but it has not shown to induce any physiological or behavioral changes for various dosages in mice and rats (19). 3-OMG has also been used to study intestinal permeability in humans. The dosages in these studies were orally given as 1 mL/kg of a solution containing 30 mg/mL of 3-OMG to pediatric patients (22,23), and up to 2.5 g in adult fasting patients along with a combination of sugars like mannitol, xylose, and lactulose (24–26).

Because of these properties, and also its accumulation in both extravascular space and cells, 3-OMG has potential to be a biocompatible contrast agent, which recently captured the interest of the CEST community. Rivlin et al showed a CEST enhancement of 15% in a mouse breast cancer model and further explored it as a contrast agent for several breast tumor models in mice of murine and human origin, varying the mode of administration and the dosage (27,28). A stroke diagnostic study of 3-OMG using the CESL technique was also performed recently (5). In addition, a spin-lock imaging study of the use of 3-OMG for 9L brain tumors has also been reported (29). In this work, we show the feasibility of using 3-OMG as a CEST agent for detecting a malignant human brain tumor (U-87 MG) in a mouse model and compare it with the previous work done on the same tumor cell type using D-glucose as an agent.

METHODS

Cell culture, tumor xenografts and animal preparation

Female severe combined immune deficiency (SCID) mice were bought from the Immune-Compromised Colony at the Johns Hopkins School of Medicine, Baltimore, MD, USA. Experiments were performed in accordance with the Johns Hopkins Institutional Animal

Care and Use Committee. U-87 MG (Uppsala 87 malignant glioma) cell lines were originally obtained from the American Type Culture Collection and grown in MEM with Earle Salts and L-glutamine (MEM 1x; Mediatech, Inc.) supplemented with 10% fetal bovine serum (Gemini Bioproducts, Inc.), 2 mmol/L sodium pyruvate (Mediatech, Inc.), 0.1 mmol/L MEM-nonessential amino acids (Mediatech, Inc.), and penicillin-streptomycin (Mediatech, Inc.). Cells were grown at 37°C in a humidified incubator with 5% CO₂. Glioma xenografts were established as previously described (30). 6- to 8-week old mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg). Orthotopic xenografts were established into the right caudate-putamen, using 1×10^5 U-87 MG cells/2 μ L for each mouse under stereotaxic control (30). Five mice were studied by MRI at 10–15 days after implanting the cells for a single dosage and three additional mice were studied for dose escalation. The mice were not fasted.

Imaging and Infusion Protocols

MRI experiments were performed on a horizontal bore 11.7 Tesla (T) Bruker Biospec system equipped with a 23-mm volume transceiver coil. During the MRI scan, mice were anesthetized by isoflurane in a mixture of O₂ and air gases and kept warm with a heating bed. The tail vein was cannulated for administration of 3-OMG, with a dead volume of 50 μ L filled with 3-OMG. A 25 G needle was used to connect the PE-60 tubing to the syringes. A 5-ml syringe with solution was positioned at a syringe pump for infusion.

An anatomical T_2 scan was performed to locate the slice with the tumor. A single slice of 1 mm thickness, with a field of view of 16×16 mm² was imaged. The complete scan protocol is shown in Figure 1. The dynamic acquisition part was performed with saturation at a single offset of 1.2 ppm with respect to water. Saturation was applied using a single continuous wave magnetization transfer (MT) prepulse of strength 1.5 μ T and duration 2 sec. The imaging part of the scan was a modified Rapid Acquisition with Relaxation Enhancement (RARE) sequence with a repetition time/echo time (TR/TE) of 5.0 s/11.15 ms, a RARE factor of 18, slice thickness 1 mm, acquisition matrix 64×64 , field of view of 20×20 mm² with two averages. The dynamic scan consisted of 96 dynamics with a temporal resolution of 10 sec. The first 3 were the S_0 images (signal intensity without saturation) followed by 15 baseline dynamics before the infusion starts. 3 g/kg (1.9 M) of 3-OMG solution prepared in phosphate-buffered saline (PBS) solvent, was infused via the tail vein at the rate of 0.2 ml/min for a period of 1 min at a time point of 3 min after the start of the dynamic acquisition scan. The total dynamic scan continued for 16 min. For the dose escalation study, we injected 6 consecutive doses of 0.5g/kg with a period of 13 minutes between successive injections. For one mouse, a volume of 0.2 ml was injected at every step. For the other two mice, a volume of 0.04 ml was injected to keep the volume similar to the single injections used for the main experiments.

We also acquired, a pre- and post-dynamic acquisition scan, a Water Saturation Shift Reference (WASSR) for B_0 homogeneity assessment (31), and a Z-spectrum (saturation spectrum of water as a function of irradiation frequency). WASSR and Z-spectra were acquired over a frequency range from –2 to 2 ppm and –6 to 6 ppm respectively with an

interval of 0.2 ppm. The saturation pulses for Z-spectra and WASSR spectra were 1.5 $\mu\text{T}/2$ sec and 0.5 $\mu\text{T}/500$ ms, respectively.

Data Analysis

All the images were normalized using the mean of the images without saturation (S_0). The first four images of the baseline saturated acquisition were discarded to assure acquiring a steady state and remove initial instabilities. A baseline image was calculated by averaging the rest of the remaining pre-injection saturated images. The dynamic difference images were obtained by subtracting each image from the baseline (3):

$$\Delta S/S_0 = S_{sat}(pre, average)/S_0 - S_{sat}/S_0 \quad [1]$$

For the dose escalation study, the image intensities were normalized to the baseline recorded before any dose was injected. The area under the curve (AUC) was calculated by taking the sum of the dynamic difference images for a given time period.

RESULTS

Figure 2 shows all images of the dynamic-contrast enhancement scan for a representative mouse. It can be seen that as the infusion of 3-OMG progresses, the vessels show enhancement first, followed by tumor and brain. Figure 3a) shows regions of interest (ROI) drawn around the tumor, a contralateral region in the brain, and an artery. The signal for the arterial input function was taken from a single voxel on the artery as indicated in the figure. These were used to obtain the corresponding dynamic curves in Figure 3b). Notice that even though the vessels became visible first in Figure 2, this was just because of the much higher 3-OMG concentration. The curve maxima are at the same time point for all three regions (due to slow infusion). The maximum enhancement, $\Delta S/S_0$, for the tumor in this example was about 6% and it stabilized to about 3% as time progressed. For 2 out of the 5 mice, no maximum enhancement followed by signal reduction and stabilization was found post-injection, but instead a gradual build-up of signal. A figure illustrating one of these is shown in the Supporting Information Figure S1. For the other three mice, we observed a similar trend as in Figure 3b), an initial signal increase towards a maximum around the end of the infusion period, followed by signal reduction and stabilization after the injection of the bolus. The enhancement in the tumor region was higher than that in the contralateral brain for all mice.

In Figure 4b), the area under the curve is shown for the last three minutes in the scan after the bolus for the mouse shown in Figures 2 and 3. While a sustained retention of 3-OMG can be seen in both tumor and brain, there is clear contrast outlining the tumor region. Figure 4c) shows the statistical analysis of the area under the curve for three set of time points: pre-infusion, 0–3 min and 8–11 min, with 0 being the start of the infusion. For all mice, the increase in the tumor signal is higher than that from the contralateral region and the contrast persists for the length of the dynamic scan as evident from the AUC points for the last three minutes. This can be seen in more detail in Figure 5a), where the average of the dynamic enhancement curves for the 5 mice is presented. The averaged curves for tumor and

brain both peaked at 80 s post-infusion. For the tumor, the maximum S/S_0 was $3.7 \pm 1.3 \%$, with S/S_0 remaining at approximately $2.5 \pm 0.6 \%$ until the end of the scan. The 3-OMG signal intensity in contralateral brain peaked to $2.5 \pm 1.1 \%$ and plateaued at $1.2 \pm 0.4 \%$. Figures 5b) show the 3-OMG weighted enhancement for all 5 mice at 60 s and 300s after the start of injection. In a previous study from our lab with the same tumor type (3) & infusion protocol, a maximum enhancement of about 1.5–3.0% and a plateau at about 1.0% after 200 s was observed when injecting D-glucose, a value about half of that observed with 3-OMG.

The shape of the averaged curves for the tumor and the contralateral brain in Figure 5a) appears similar to that in Figure 3, indicating that the large initial enhancement in two mice dominated the averaging.

In order to check the minimal dose needed for detectable signal and the linearity of the CEST effect with concentration, we tried to study different mice with different concentrations, but due to tumor inhomogeneity this gave inconsistent results. We therefore instead performed a dose-escalation study on 3 mice. For one mouse a volume of 0.2 ml (0.5g/kg) was injected for each increment and the contrast between the tumor and the contralateral brain is shown in the Supporting Information Figure S2a. After the second dose accounting to the total of 1.0g/kg, the tumor-brain contrast reached about 1%, while it reached about 3% with a total dosage of 3g/kg, with a nearly linear trend of 25% increase in every step. However, this study can be criticized due to a large increase in liquid in the vasculature. Therefore, for two mice, we injected 6 incrementing doses of 0.5g/kg of volume 0.04 ml each with a period of 13 minutes between successive injections. This maintains the total dose of 3g/kg while the total volume injected in the mice during the course of this scan (0.240 ml) was comparable to the volume injected individually in the single-injection studies. Both mice showed similar results and the average (tumor-brain) contrast after baseline correction is shown in Supporting Information Figure S2b. The tumor-brain contrast started appearing at a total dose of 1.5–2g/kg.

As previously pointed out (3), the single-frequency DGE difference signal includes the effects of both CEST and T_2 relaxation, which add up. In order to get an impression of the T_2 contribution, we compared the saturation linewidths of the pre- and post-infusion WASSR scans of the tumor and the contralateral brain (Supporting Information Figure S3). Since the enhancement is long-lasting, this should be a proper approach. We did not observe any difference in these pre- and post-line widths for any region indicating at most a minor contribution of T_2 .

DISCUSSION

These results show that similar to D-glucose, 3-OMG can also be used as a contrast agent for brain tumors and its uptake studied by dynamic CEST-MRI studies. In tumors where the blood brain barrier (BBB) is disrupted, the gadolinium-based contrast agents diffuse from the intravascular to the extracellular space. In addition to diffusion over the disturbed BBB, D-glucose and 3-OMG also get transported into the cell via the regular mechanism of facilitated diffusion by the hexose carriers in the brain, i.e. the glucose transporters GLUT-1 and 3. For D-glucose, the amount of free glucose in the organ is affected by: a) metabolism

of glucose within the cells, leading to lower concentration of free glucose to be detected by CEST and b) the insulin response to high glucose concentration in the blood which limits the available free glucose in the blood. With 3-OMG, we circumvent these two issues, leading to increased CEST signal (20,32).

Based on measurements of phosphorylated glucose in the brain to be in the low micro-molar range (33), it is a good assumption that the D-glucose-weighted CEST enhancement comes from the plasma and the extra-cellular extravascular space (EES) (3). Relative to D-glucose, 3-OMG has a 90% affinity to the hexose carriers in the mouse brain (34) and is known to compete with glucose for access to these transporters (35–37). Insulin also has been found to not inhibit the bidirectional transport of 3-OMG through the carriers (15,38–40). Since 3-OMG is not metabolized, it gets trapped after entering the cells until an equilibrium is established between the blood and the tissue and is then gradually removed. Thus, increased CEST enhancement in 3-OMG weighted images relative to the D-glucose weighted images most likely comes from the intercellular space. While this is the case for both brain cells and tumor cells, we also found an increased contrast between brain and tumor for 3-OMG relative to D-glucose. One possible reason is that many cancer cell lines have an increased expression of glucose transporters (37). In the U-87 tumor line, it has been reported that GLUT-3 are overexpressed which is the largest transporter in the brain with a 5-times greater transport capacity (41). This increased GLUT-3 expression in tumor areas as compared to the normal brain provides a compelling reason for the improved contrast between the brain and the tumor for 3-OMG versus D-glucose.

In $T_{1\rho}$ studies of OMG, it was noticed that there was an additional role of osmolality to the transverse contribution of the relaxivity (5). Due to the high osmolality of the blood in the first few minutes after infusion, water moves from the extravascular to the intravascular spaces. Since the transverse relaxivity also contributes in CEST experiments through a broadening of the direct water saturation and thus an apparent increase in the dynamic enhancement, such effects may also contribute to the curves in Figure 3b, leading to a maximum within a few seconds of the injection.

When using 3-OMG with the same dose as in our experiment to study a breast tumor model, an enhancement of nearly three times (~9.5%) was reported after an hour of infusion (28). These investigators utilized three modes of administration of 3-OMG: intravenously (IV), intraperitoneally (IP), and per os (PO) and found nearly similar enhancement in their most aggressive breast tumor model irrespective of the method of administration. PO, being the least invasive method of all, can be a suitable mode of administration, but for brain tumors until a detailed comparative study is performed, IV can be used for diagnosis because of faster access of the agent to the brain.

Another significant diagnostic usage of 3-OMG is as a contrast agent for stroke imaging as shown recently by Jin et al (5). They showed how the 3-OMG induced difference in $T_{1\rho}$ can help in differentiating the ischemic core from hypo-perfused tissues at risk and normal tissues. In ischemia, any form of D-glucose treatment/infusion can be an impediment to recovery due to lactate generation, which 3-OMG avoids and inhibits due to its dominance in occupation of the transporters. Therefore, it may act as a useful alternative if D-glucose

infusion cannot be used. One caveat however is that a high dose of 3-OMG induces a systemic increase in plasma glucose concentration few hours after its injection (42). During this period of excess of 3-OMG in the plasma, the energy demands are not met by the glycolytic support mechanism, instead a glycolytic stress response is triggered, leading to a general hyperglycemia.

CONCLUSIONS

We showed that 3-O-methyl glucose can, similar to D-glucose, act as a CEST contrast agent for brain-tumor detection. Further work is needed for translating it beyond mouse models, as detailed toxicity research on 3-OMG infusion is not available. Given the use of oral 3-OMG as a marker for nutrient absorption studies in human, 3-OMG has potential as a safe MRI contrast agent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

Experimental scheme employed to collect the images. An anatomical image was recorded first, followed by WASSR and a Z-spectrum. Dynamic glucose enhancement acquisition is one continuous scan with 96 dynamics lasting 16 min, including a 1 min infusion. The first and the last few dynamics are S_0 . The three regions showed with red arrows indicate the periods analyzed in Figure 4.

Figure 2:

Anatomical image (top left) followed by the dynamic enhancement difference images (pre-injection average – image), of 10 s each for a representative mouse. The box labeled infusion shows the images during the period of infusion. The color bar represents the absolute value of the percentage change of the water signal ($\Delta S/S_0$).

Figure 3:

a) Shows the regions of interest taken over the tumor, the contralateral part of the brain, and the vessel of the mouse shown in Figure 2. **b)** shows the dynamic time curves for tumor, contralateral sides of the brain and an arterial input function. The inset shows the tumor and contralateral brain curves at enlarged scale.

Figure 4:

a) Anatomical image of the mouse shown in Figure 2. **b)** The area under the curve image calculated for the last three minutes of the scan, a period of 8–11 min post injection of 3-OMG. **c)** AUC statistics for the 5 mice for three periods: pre-infusion, 0–3 minutes from the start of infusion, and 8–11 min post infusion. The statistical analysis was calculated using the paired student's t-test with $P < 0.05$.

Figure 5:

a) Averaged dynamic enhancement curve with the error bars representing the standard deviation, for the tumor and the contralateral brain of 5 mice. The $\Delta S/S_0$ values for all the mice at b) 60 s and 300 s post injection, show that tumor-brain contrast is evident in all mice. The statistical analysis was calculated using the paired student's t-test with $P < 0.05$.