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Noninvasive photoacoustic computed tomography of mouse brain metabolism *in vivo*

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ABSTRACT

We have demonstrated the feasibility of imaging mouse brain metabolism using photoacoustic computed tomography (PACT), a fast, noninvasive and functional imaging modality with optical contrast and acoustic resolution. Brain responses to forepaw stimulations were imaged transdermally and transcranially. 2-NBDG, which diffuses well across the blood–brain-barrier, provided exogenous contrast for photoacoustic imaging of glucose response. Concurrently, hemoglobin provided endogenous contrast for photoacoustic imaging of hemodynamic response. Glucose and hemodynamic responses were quantitatively decoupled by using two-wavelength measurements. We found that glucose uptake and blood perfusion around the somatosensory region of the contralateral hemisphere were both increased by stimulations, indicating elevated neuron activity. While the glucose response area was more homogenous and confined within the somatosensory region, the hemodynamic response area had a clear vascular pattern and spread wider than the somatosensory region. Our results demonstrate that 2-NBDG-enhanced PACT is a promising tool for noninvasive studies of brain metabolism.

Introduction

In mammals, the brain performs numerous computation-intensive tasks such as information processing, perception, motor control and learning, and thus consumes a large amount of energy in proportion to its volume. For example, humans devote 20–25% of their metabolism to the brain, where the energy is mostly used for sustaining the electric charge (membrane potential) of neurons (Mink et al., 1981). In humans and many other species, the brain gets most of its energy from oxygen-dependent metabolism of glucose (Coghill et al., 1994; Fox and Raichle, 1986; Gjedde et al., 2002; Hall et al., 2012; Smith et al., 2009). An abnormal metabolic rate of glucose and/or oxygen usually reflects a diseased status of the brain, such as cancer or Alzheimer's disease (Fulham et al., 1992; Mosconi et al., 2009). In addition, physiologically active regions of the cerebral cortex consume more energy than inactive regions (Raichle and Gusnard, 2002). These phenomena have formed the basis for functional brain imaging methods, including positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (Mehagnoul-Schipper et al., 2002; Schlemmer et al., 2008).

However, PET depends on the administration of radioactively-labeled tracers (e.g., 2-deoxy-2-fluoro-D-glucose, FDG), a complex procedure with exposure to ionizing radiation. fMRI is primarily sensitive to deoxy-hemoglobin and suffers from slow imaging speed. Moreover, both PET and fMRI are expensive techniques with poor spatial resolutions. Therefore, a fast, noninvasive and non-ionizing imaging modality with good spatial resolutions is needed to advance metabolism-associated studies of brain physiology and pathology.

On the basis of the photoacoustic effect, photoacoustic tomography (PAT) solves the resolution drawback of pure optical imaging and the contrast drawback of pure ultrasonic imaging (Wang and Hu, 2012; Wang, 2008, 2009a; Yao and Wang, 2011). In PAT, photon energy absorbed by molecules is partially or completely converted into heat, which thermoelastically induces pressure waves. The induced pressure waves are detected by ultrasonic detectors to form an image (Wang and Hu, 2012). PAT is capable of anatomical, functional, molecular and metabolic imaging of small animals, with highly scalable spatial resolution and penetration depth (Wang, 2009a, 2009b; Yao and Wang, 2011; Zhang et al., 2006). Photoacoustic computed tomography (PACT) is a major implementation of PAT, which aims at fast data acquisition, sub-millimeter resolution and deep penetration depth beyond the optical diffusion limit (Li et al., 2008, 2010; Wang et al., 2003; Xia et al., 2011). Noninvasive, label-free and functional PACT of the rat brain was demonstrated by accurately mapping brain lesions and cerebral hemodynamics (Wang et al., 2003). Molecular imaging of a mouse brain tumor *in vivo* was also

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performed with PACT, using IRDye-800-c as the contrast agent (Li et al., 2008).

Here, for the first time, we have demonstrated that PACT is able to image glucose uptake in the mouse brain, using a newly developed glucose analog 2-deoxy-2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-D-glucopyranose (2-NBDG). To demonstrate the metabolic imaging capability of PACT, we studied *in vivo* forepaw stimulation responses. Our phantom and animal studies showed that PACT could spectrally separate 2-NBDG and blood using two-wavelength measurements, thus decouple the glucose and hemodynamic responses to the stimulations. Open-scalp photoacoustic microscopy and fluorescence imaging were used to validate the results from PACT.

Materials and methods

2-NBDG

2-NBDG is a newly developed fluorescent 2-deoxyglucose (2-DG) analog (Bem et al., 2007; Cheng et al., 2006; Gaudreault et al., 2008; Itoh et al., 2004; Langsner et al., 2011; Millon et al., 2011; O'Neil et al., 2005; Sheth et al., 2009; Tsytsarev et al., 2012). Like the FDG (molecular weight: 181) used in PET studies, 2-NBDG is transported into cells via the same GLUT as glucose (Sheth et al., 2009). Once taken up by the cells, 2-NBDG is phosphorylated to 2-NBDG-6-P, which prevents it from being released again from the cells. However, because of the lack of the 2-hydroxyl group needed for glycolysis, 2-NBDG-6-P cannot be further metabolized. Therefore, the distribution of trapped 2-NBDG is a good reflection of glucose metabolism (O'Neil et al., 2005). Because 2-NBDG is a relatively small molecule (molecular weight: 342) [Fig. 1a], it crosses the blood–brain-barrier much more easily than other near-infrared fluorophore-labeled 2-DG analogs, such as IRDye800-DG (molecular weight: 1330) (Cheng et al., 2006; Itoh et al., 2004). Moreover, 2-NBDG has its peak absorption at 478 nm, where hemoglobin has a much lower absorption than at the peak absorbing wavelength [Fig. 1b]. As a result, the signal contribution from hemoglobin can be neglected at this wavelength. These features have made 2-NBDG particularly suitable for brain studies.

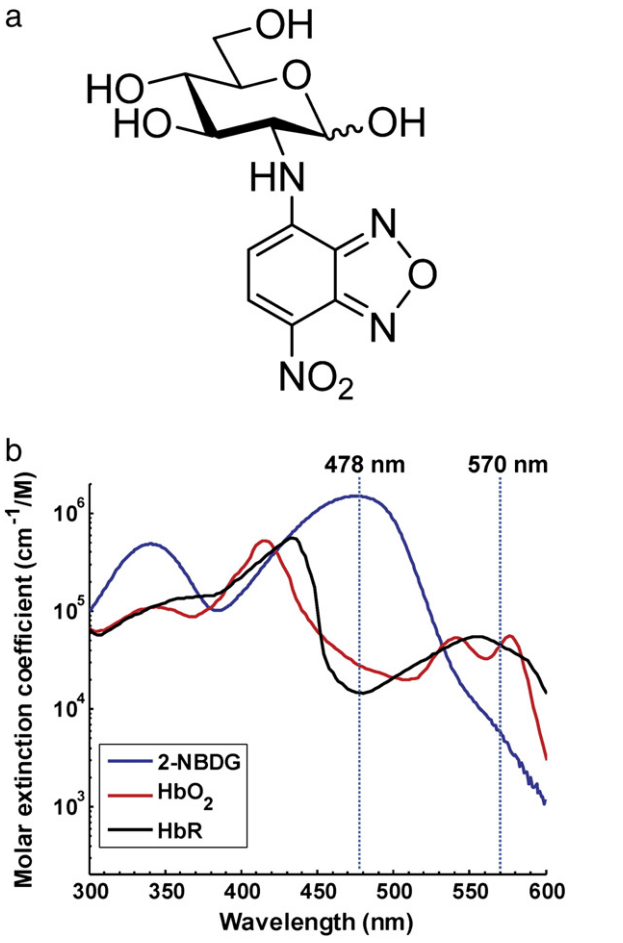

Fig. 1. 2-NBDG ($C_{12}H_{14}N_4O_8$). (a) Chemical structure. (b) Molar extinction spectra of 2-NBDG, deoxy- and oxy-hemoglobin.

Noninvasive photoacoustic computed tomography (PACT)

Fig. 2a is the schematic of the PACT setup. An OPO laser (BasiScan 120, Spectra-Physics) is pumped by an Nd:YAG laser (Brilliant B, Quantel) with a third harmonic generator (355 nm) to provide pulses with wavelengths tunable from 420 nm to 680 nm. The pulse duration is 6 ns, and the pulse repetition rate is 10 Hz. The laser beam is homogenized by an optical diffuser (EDC-5, RPC Photonics) to provide uniform illumination over the mouse brain. The maximum light intensity at the tissue surface is approximately 10 mJ/cm^2, below the ANSI limit at the chosen wavelengths. The photoacoustic signals are detected by a 5 cm diameter full-ring ultrasonic transducer array with 512 elements (Imasonic, Inc.). The central frequency of the ultrasonic transducer array is 5 MHz, and the 6-dB bandwidth is more than 80%. Each element in the array is directly shaped into an arc to produce an axial focal depth of 19 mm. The combined foci of all elements form a relatively uniform imaging region of 20 mm diameter and 1 mm thickness. Within this region, the axial (radial) resolution is 0.10 mm, and the transverse (tangential) resolution is 0.25 mm (Gamelin et al., 2009). As shown in Fig. 2b, after a complete data acquisition from all 512 elements, the raw data is reconstructed to form a photoacoustic image of the brain based on the universal back-projection algorithm (Xu and Wang, 2007). The imaging speed of the current system is 1.6 s per frame.

Optical-resolution photoacoustic microscopy (OR-PAM)

OR-PAM, another implementation of PAT, aims at capillary-level resolution within the optical diffusion limit (Hu et al., 2011; Maslov et al., 2008; Yao and Wang, 2010, 2011; Yao et al., 2009, 2010, 2011). As shown in Fig. 2c, by focusing the laser pulses to a diffraction-limit spot using an objective with an NA of 0.1 (AC127-050-A, Thorlabs), OR-PAM achieves a transverse resolution of 5 μm. By using a single-element ultrasonic transducer with a central frequency of 50 MHz and a 6-dB bandwidth of 100% (V214-BB-RM, Olympus-NDT), OR-PAM achieves an axial resolution of 15 μm. Due to scattering by the tissue, the penetration depth of OR-PAM is limited to ~1 mm, which is sufficient for transcranial imaging of the cerebral cortex but not transdermal imaging. The arrival time of the PA signal provides depth information, and volumetric imaging is acquired by two-dimensional raster scanning of the sample. In this study, to validate the cortical vascular image obtained by PACT, the same mouse was imaged by OR-PAM at 570 nm after the PACT imaging. The scalp was surgically removed, while the skull was left intact. It took about 30 min to acquire an OR-PAM image over a 5×10 mm^2 area.

Fluorescence imaging

To confirm the stimulation induced changes in 2-NBDG uptake in the brain, open-scalp fluorescence imaging using the same