Significant BOLD Signal Reduction Induced by Perfluorocarbon Emulsion in the Rat Brain

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Introduction: Perfluorocarbon (PFC) emulsion, an artificial oxygen carrier has been used as "oxygen therapeutics" to treat diseases with compromised oxygenation such as ischemia, air embolism and trauma[1-5]. In addition, when mixed with $^{17}O_2$, it can be used as a blood carrier of ^{17}O-labeled oxygen isotopes for measuring the oxygen consumption rate using a sophisticated MR imaging mthod[6]. It is known that the Blood-Oxygen-Level Dependence (BOLD) functional MRI (fMRI) is based on the endogenous deoxygenated hemoglobin (dHb) concentration change induced MR signal changes. Since the PFC emulsion can greatly enhance the oxygen solubility in the blood, will it shift the concentration ratio between dHb and oxyhemoglobin (HbO_2), thus, influence the BOLD signal? If it could, will BOLD increase or decrease and how much this change will be? Intrigued by these questions, we introduced PFC emulsion to normal rats and collected a serial of the rat brain BOLD images in this study. Meanwhile, total blood hemoglobin (THb), oxyhemoglobin saturation (%HbO_2), pH and oxygen partial pressure (PO_2) in both artery and venous bloods were sequentially measured for understanding the BOLD change caused by PFC emulsion.

Material and MRI method: Nine rats (449±35g) under 2% isoflurane anesthesia were used in this study. Both arterial and venous blood samples were taken from the rats before and after the artificial blood emulsion (OxyToT, Rockland Technimed Ltd; 0.01ml/g dose, injected over 10 minutes period). THb and %HbO_2 were measured with AVOXimeter (Int Tech Corp) while pH and PO_2 with blood gas analyzer (Siemens). One rat underwent bench study only and the rest of rats (n=8) were used for both MRI experiments and blood analysis. MRI measurements were performed using a 9.4T/31cm magnet interfaced with VNMRJ consoles (Varian) and a ^1H surface coil (2.8cm×2cm). Gradient echo EPI (TR=1sec; TE=17ms; FOV=3.2×3.2cm; matrix=64×64; thickness=2 mm) was used for imaging BOLD signals. Anatomic images were acquired with a Fast Spin Echo sequence (TE=10ms; TR=4sec; FOV=3.2×3.2cm; matrix=128×128; thickness=2 mm; 8 echo train length). MRI data analysis was performed using the Matlab software package. Region of interest (ROI) of somatosensory cortex were used to quantify the relative BOLD (rBOLD) change ($=SI_{\text{after injection}}/SI_{\text{before injection}}$) during and after the PFC injection. Finally, the rBOLD maps were overlapped on the anatomic image. The relative arterial THb, %HbO_2, pH and PO_2 changes after the injection of PFC emulsion are normalized by their baseline values then averaged among 9 rats at 8.3, 37.1,114 and 173.5 minutes for arterial blood samples, and at 11.7, 37.3, 98.3 and 169.3 minutes for venous blood samples.

Results: Figure 1 shows that the %HbO_2 level remained constant in the arterial blood during the entire experiment (~170 minutes) after the PFC emulsion injection; in contrast, it dropped significantly in the venous blood to only 64% at end of the experiment. A smaller decrease (10%) of THb was observed during the initial 40 minuets following the PFC injection but gradually diminishing in both arterial (≤5%) and venous (≤2%) blood, which might be due to multiple blood drawings within a short time period. In addition, PO_2 level in the artery was relatively stable except for a 16% increase following the PFC injection, while a 10% decrease in the venous blood was observed after the PFC injection which reduced to ~17% PO_2 reduction in latter half of the experiment. The pH values in all blood samples remained constant. The BOLD signal in the rat brain cortex decreased to 96.6±0.8%, 95.4±1.9%, 94.3±2.1% and 92.5±1.3% (n=8) of the baseline at 10, 60, 90 and 160 minutes, respectively, after the PFC emulsion injection. This trend in decreased BOLD signal coincidently correlated well with the declined %HbO_2 level measured in the venous blood samples (correlation coefficient = 0.94). Figure 2 shows the rat brain anatomic image (a) and decreased BOLD images (120 volume or 2 minutes average) measured at 10, 90 and 160 minutes (b,c,d) after the injection of PFC emulsion in a representative rat.

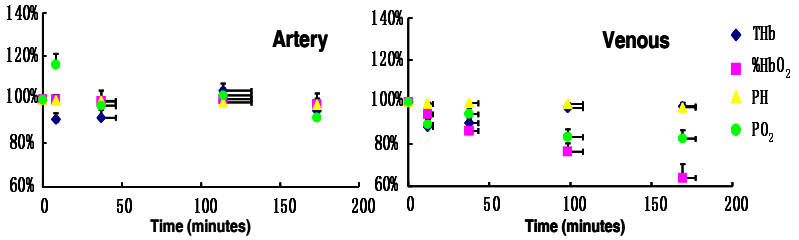

Figure 1. Time courses of normalized artery and venous Thb, %HbO_2, PH and PO_2 after PFC emulsion injection (n=9).

Discussion: The magnetic susceptibility difference between HbO_2 and dHb is the physical cornerstone of the fMRI technique. Specifically, dHb is more paramagnetic than HbO_2, thus leads to magnetization de-phasing and EPI signal loss. The BOLD signal increase observed in most fMRI studies results from the dHb concentration decrease (interplays among Cerebral Blood Flow (CBF), Cerebral Blood Volume (CBV) and Cerebral Metabolic Rate of Oxygen ($CMRO_2$)) within an imaging voxel in response to brain stimulation. The decreased BOLD signal in the rat brain cortex after the injection of the PFC emulsion observed in the present study indicates an increased dHb concentration in the venous blood compared to the control state, which is consistent with the %HbO_2 change measured in the vein. Since the rats were in a resting state without any external stimulation, $CMRO_2$ is likely unchanged. Therefore, the increased dHb concentration must be related to the hemodynamic changes caused by the introduction of PFC emulsion. Although the CBF and CO_2 solubility have been found increased after injection of PFC emulsion[7], it is hard to explain why BOLD signal decreased and the dHb increased (i.e., %HbO_2 decreased) in the venous blood in this study. One possible explanation is that HbO_2 in the venous is forced to release oxygen and is converted to dHb due to the high dissolvability of oxygen enhanced by PFC. In another word, oxyhemoglobin loses oxygen due to the competition with PFC when the oxygen is limited in the venous blood. This does not happen in the artery because there is more than enough oxygen available after the blood passes through the pulmonary circulation. The venous PO_2 level decrease after the PFC injection can also be explained by the strong adsorption of oxygen to the PFC and these adsorbed oxygen molecules might not be easily detected by the blood gas analyzer. The images in Figure 2 show a greater BOLD signal drop at the brain cortex and the venous sinus than the rest of the brain, which might be due to the higher density of the vascular and/or neurons in the cortex and drainage collection of venous blood in the sinus. Note the spatial pattern of the decreased BOLD correlates reasonably well with the perfusion distribution shown in the CBF map, suggesting again that the observed BOLD change is related to the vascular hemodynamic changes caused by the PFC emulsion injection. The significance of this study manifests on two aspects. First, PFC emulsion potentially can be used as an fMRI BOLD contrast agent reflecting the concentration change of venous dHb. This property offers a greater advantage over the traditional contrast agents because it can be used for the treatment and assessment of the tissue function at the same time. Secondly, caution needs to be exercised when performing a $CMRO_2$ imaging study using the indirect ^1H MRI approach and the $^{17}O_2$ carried by PFC emulsion since it could introduce a large baseline drift of water proton signal, which last at least 170-minute after the PFC injection.

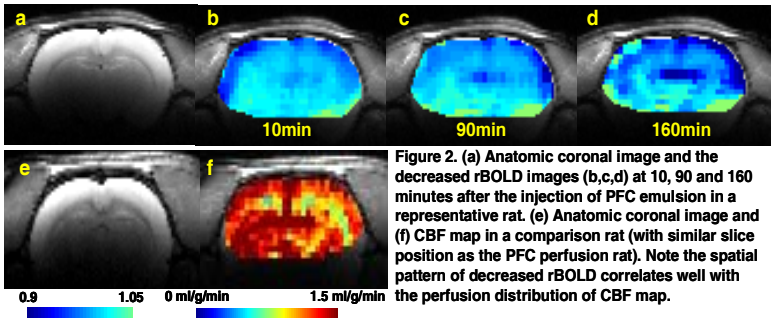

Figure 2. (a) Anatomic coronal image and the decreased rBOLD images (b,c,d) at 10, 90 and 160 minutes after the injection of PFC emulsion in a representative rat. (e) Anatomic coronal image and (f) CBF map in a comparison rat (with similar slice position as the PFC perfusion rat). Note the spatial pattern of decreased rBOLD correlates well with the perfusion distribution of CBF map.

Conclusion: In summary, we have shown that the injection of PFC emulsion to the normal rat could induce both %HbO_2 decreasing (up to 36% at 170 minutes after the injection) in vein and BOLD decreasing (up to 7.5% at 160 minutes injection) in the rat brain. These findings might be related to the competition of oxygen bound between PFC and oxyhemoglobin in vein, results in the conversion of HbO_2 to dHb. Therefore, PFC emulsion injection could potentially serve the treatment and the functional assessment purposes at the same time.

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References: 1. Arnold JV et. al., *Perfusion*, 1993; 2**.** Aleksandrin VV et.al *Anestheziol Reanimatol*, 2003; 3. Lynch PR et.al *Undersea Biomed Res*, 2005; 4**.** Mahon RT et.al *Aviat Space Environ Med*, 2006; 5. Kwon TH et al. *J Neruosurg*, 2005; 6. Kyle SM et al. *MRM*, 2010; 7. Bruce. D et al. *J Appl Physio*, 2009.