Improved mapping of extracellular pH in C6 gliomas by ^{1}H MRSI shows low correlation between Lactate concentration and pHe changes induced by infusion of glucose

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Introduction

Extracellular pH (pH_e) is more acid in tumors than in normal tissue and shows a very heterogeneous distribution within the tumor. Because most tumors exhibit a characteristic high glycolytic metabolism, it became widely accepted that this is the main cause of tumor acidity. However, the relationship between both processes *in vivo* remains poorly investigated. In the last decade new methods have been developed that allow the pH to be measured *in vivo* [1]. In a previous work, using one of these new methodologies, we reported that no correlation between pH and lactate concentration could be found *in vivo* in C6 gliomas [2]. In the current communication we further investigated the correlation between glycolytic metabolism and tumor acidity by looking at the changes in lactate concentration and pH_e produced by glucose infusion. We used a new and more sensitive pH probe, ISUCA, as well as improved spectroscopic imaging methods.

Methods

Tumor model. Orthotopic gliomas were induced by stereotaxic injection of 10^5 C6 cells in Wistar rats weighing 200-250 g. MR experiments were performed 3-4 weeks after implantation. *Animal preparation.* Rats were anesthetized with a mixture of 1-1.5% (v/v) isoflurane and 30% O_2. The femoral vein was catheterized for pH indicator and glucose infusion. *In vivo MR.* All the experiments were performed on a 7T magnet, controlled by a SMIS console. The spectroscopic imaging sequence consisted of a water suppression module (VAPOR) followed by a PRESS sequence and spiral readout gradients. For metabolite images, the echo time was 136ms, the spiral readout module consisted of 8 out-and-in spirals of 8 turns each, with 32 temporal interleaves and 2 spatial interleaves. For pH imaging, the echo time was 40 ms, the spiral readout module consisted of 8 out-and-in spirals of 4 turns each, with 32 temporal interleaves and 4 spatial interleaves [3]. A metabolite image and a short echo time reference image were acquired in a PRESS volume including the tumor. ISUCA was infused (1 M, 6 mL/h during 20 min then 1.2 mL/h). After 30 min, two successive pHe images were acquried. Glucose was then infused (1 M, 1.2 mL/h) during 50 minutes. An ISUCA image and a metabolites image were acquired 25 min after the beginning of the infusion. After data resampling, filtering, zero-filling and FFT, spectroscopic images were obtained.

Results

By integrating the peaks in the 136 ms echo time image in each voxel, maps were reconstructed for the main brain metabolites observable *in vivo*, i. e., choline, creatine, NAA and lactate. In the 40ms echo time image, the position of the ISUCA peak in each voxel was used to generate pH_e maps of the tumor (Fig. 1a). Mean pH_e was 7.01 ± 0.006 (SEM) and 6.90 ± 0.004 before and after glucose infusion, respectively. The statistical analysis revealed significant changes in both pH_e and lactate concentration in response to glucose infusion. However, a voxel by voxel comparison of Δ[Lactate] and ΔpH_e showed a very low correlation between both processes (Fig. 1b).

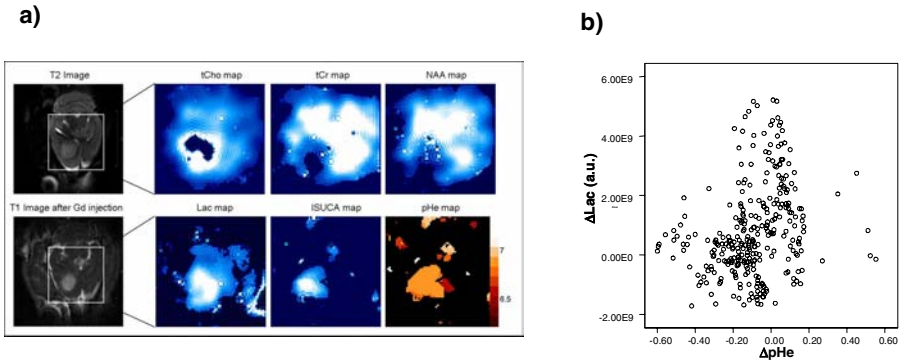

Figure 1. a) The anatomic image of a representative tumor and the corresponding maps of metabolites, ISUCA, pHe and GdDOTA. b) Correlation between changes in pHe and Lac in response to glucose infusion.

Discussion

The extracellular pH of C6 gliomas was measured employing a new pH indicator with improved sensitivity, ISUCA, and new spectroscopic methodology that allowed pH maps to be generated within 25 minutes. The infusion of glucose induced a decrease on pH of 0.12 units. Concomitant to this change, an increase in lactate was observed. However, the correlation analysis revealed that both events are **not** tightly correlated, indicating that processes other than lactate metabolism have to be taken into account in order to explain the pH variations observed *in vivo*.

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