High Resolution pHe Imaging of Rat Glioma using pH-dependent relaxivity

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Introduction. The extracellular/interstitial pH (pH_e) of both human and animal tumors is acidic as shown by ^{31}P and ^{1}H spectroscopic techniques (1-3). Tumor acidity plays a crucial role in tumor progression and invasion, as well as in resistance to therapy (4, 5). pH measurements carried out using spectroscopic methods showed heterogeneous distribution of pH_e within the tumors. However, because of the poor spatiotemporal resolution inherent to these techniques, the causes of this heterogeneity remain poorly understood. New methods based on pH-sensitive T_1 relaxivity are attractive both because of improved spatiotemporal resolution and also because they are promising candidates for clinical use. In the current study, we use the pH-sensitive T_1 relaxation contrast reagent (CR), GdDOTA-4AmP (6) to generate high resolution pH_e maps of C6 gliomas implanted in rats. The concentration of the CR was determined by comparison to the pH-insensitive CR, GdDOTP, which shows identical pharmacokinetics (PK). Time to the maximal intensity (TMI) maps were also generated and compared to pH_e. The results obtained in this work show that pH_e of C6 gliomas is acidic and heterogeneous, and it is correlated to tumor vascularity, this is, pHe is less acidic in those areas of the tumor that show better perfusion and/or vascular permeability.

Methods. *Glioma model.* C6 glioma cells (10^5) were injected stereotaxically in the right caudate nucleus of rats weighing 200-250 g. After 2 to 3 weeks gliomas occupied 30-50% of the right hemisphere. *Animal preparation.* Rats were anesthetized with a mixture of 1-1.5% (v/v) isoflurane and O_2. Body temperature was maintained at 36.5-37.5°C and monitored during all the *in vivo* experiments using a rectal fluoroptic probe. The tail vein was cannulated for CR delivery. *In vivo MRI.* All the experiments were performed on a Bruker Biospec 4.7 T system. Both proton density (TR=8000 ms, TE= 6.9 ms) and T1 weighted (TR=80 ms, TE=6.9 ms) spin echo images were acquired previous to any CR injection. Then, 0.1 mmol/kg of the pH-insensitive CR GdDOTP was administered through the tail vein and T_1-weighted images were acquired for one hour. Following this first bolus, the same protocol was repeated using either the same CR ($GdDOTP^{5-}$) or GdDOTA-4AmP (pH-sensitive). Time to the maximal intensity maps were generated for both PK time courses. PK reproducibility was evaluated by comparing the TMI maps (Fig. 1a, 1b) as well as averaged timecourses from ROIs within the tumor (not shown). In-plane shifts due to motion were estimated and corrected by generating one or two pixels shifted images in all directions and performing correlation analysis of the corresponding TMI maps. Then pH_e was calculated for each pixel as previously described (7). *In vitro* relaxivities were measured in fetal bovine serum to estimate the effect of proteins on the relaxivity properties of both CRs.

Results and Discussion. GdDOTP and GdDOTA-4AmP show similar pharmacokinetics *in vivo* as can be inferred from the TMI maps (Fig. 1a, 1b) and the TMI histograms (Fig. 1d). Therefore, the distribution of GdDOTP was used to correct for the concentration of GdDOTA-4AmP on a pixel-by-pixel basis, subsequently allowing for the generation of pHe maps (Fig. 1c). The pHe of C6 gliomas was found to be acidic and heterogeneous. In the example shown in Fig. 1 the average pH_e was 6.86 and the standard deviation 0.43, which is in good agreement with previous studies using ^{1}H MRSI in this same model system (3). The correlation analysis between pHe and TMI showed a negative and statistically significant dependence. The TMI depends on both tumor perfusion and permeability. Hence, its negative correlation with pH_e (Figure 1e) indicates that tumors are more acidic in less well perfused and/or permeable parts of the tumor. Interestingly, pixels with very low perfusion (long TMI) can be alkaline, which probably corresponds to necrosis.

Conclusions. The current investigation demonstrates the feasibility of generating high resolution pH_e maps in tumors using pH-sensitive T_1 relaxation CRs. It also demonstrates that the dual injection method based on the use of a pH-insensitive CR to determine the concentration of the pH-dependent one can be applied to tumors. However, because of the slow washout of the CRs from tumors (cf. kidneys, ref. 7), the experimental time is too long to be suitable for clinical use. Nonetheless, the current method is useful for model organisms and can be used to demonstrate heretofore undocumented relationships between pH_e and perfusion.

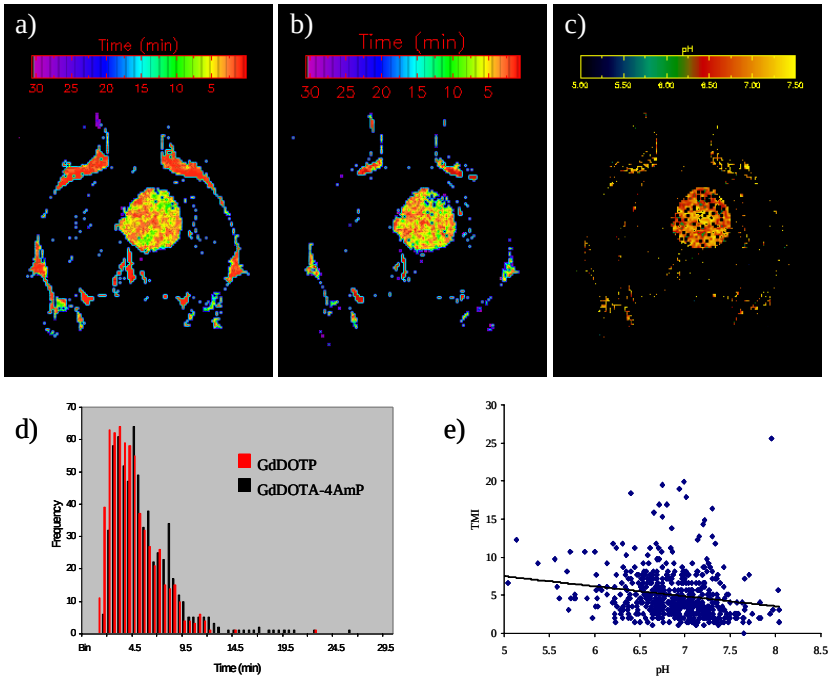

Figure 1. a) time to the maximal intensity map of $GdDOTP^{5-}$, b) time to the maximal intensity map of $GdDOTP\text{-}4AmP^{5-}$, c) pHe map (Mean =6.86 Std. Dev. =0.43) , d) histograms of time to the maximal intensity of GdDOTP and GdDOTA-4AmP in the tumor, e) Pearson correlation analysis of pH_e vs. time to maximal intensity. Correlation coefficient = -0.182 ($P<0.001$).

References

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