Distribution of Steady State Contrast Agent Concentration and Interstitial Fluid Pressure in Human Xenografts

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Introduction

Many solid tumors show an increased interstitial fluid pressure (IFP), which forms a barrier to transcapillary transport. This barrier leads to an inefficient uptake of therapeutic agents and hence, demonstrates a physical resistance to therapy. Currently IFP is measured locally by invasive methods that may damage the tissue and modulate the IFP level. Herein we show a non-invasive method to map the distribution of IFP in tumors.

Methods

Studies were performed in two different experimental tumor models: 1. Human H-460 non small cell lung carcinoma cells inoculated subcutaneously in the flank of nude mice. 2. Human MCF7 breast cancer cells inoculated in the mammary gland of nude mice. The mice were anesthetized throughout the experiments by exposure to 1% isoflurane in an O_2/N_20 (3:7) mixture. All animal procedures were approved by IACUC.

IFP in the tumors and other normal tissues was measured by the wick-in-needle method (1).

Histological characterization included H&E staining for morphological characterization and anti CD31 immunostaining of endothelial cells in order to trace the capillary distribution in the tumors.

The images were acquired with a 4.7 T Biospec spectrometer (Bruker). The MRI protocol included T1 measurements by fast SNAP inversion recovery (IR) sequence with a non selective inversion pulse, inversion time, ranging from 50 to 10000 msec and fast low angle GE acquisition with TE/TR=3.5/36.7 msec, matrix size 256:256, and FOV 4 cm.

GdDTPA was administered by slow infusion through the tail vein at a rate of 0.67 mmol/ kg/h during 2 hours. At 20 min of infusion GdDTPA reached steady state concentrations in the plasma, namely the rate of infusion was equal to the rate of clearance from the blood. Following blood steady state, the other tissues also reached steady state concentration, namely constant concentration in the interstitial space, ~equals to the plasma concentration.

T1 relaxation measurements were performed before infusion and at 100 min of infusion at steady state.

The T1 measurements served to assess the tissue GdDTPA concentration per pixel [Ct] according to: $1/T1 = 1/T10 + r1[Ct]$ where r1 is the T1 relaxivity = 4.3 $sec^{-1}mM^{-1}$(2). Steady state extracellular GdDTPA concentration maps were calculated by dividing the Tissue GdDTPA concentration by an average extracellular volume fraction (EVF) of 0.2 for H-460 tumors and 0.4 for MCF7 tumors. These values were assessed from histopathology and previously determined diffusion MRI (3) (4).

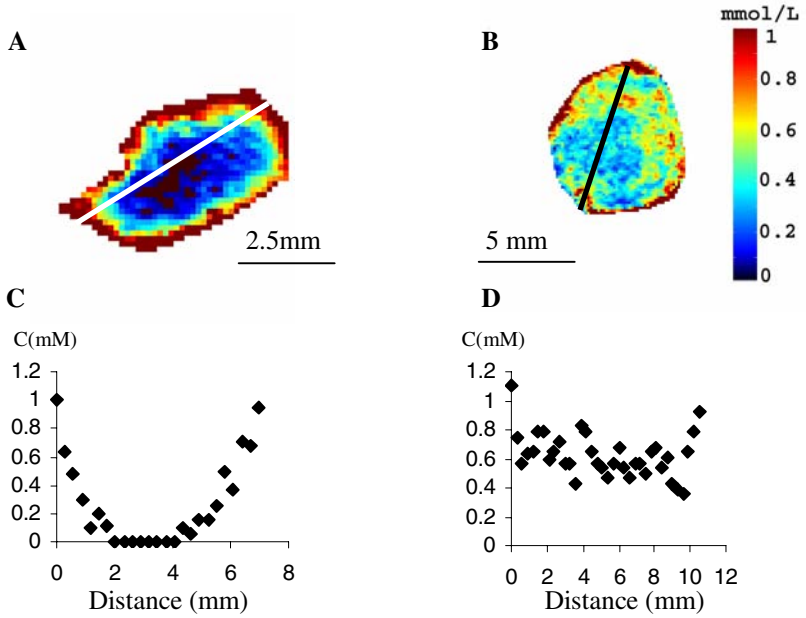

Figure 1. GdDTPA extracellular concentration maps and profiles in H460 and MCF7 tumors: Extracellular concentration map of H460 (A) and MCF7 (B) tumors (C,D) Profiles of the extracellular concentration along the lines drawn in A and B.

Results

IFP measurements, applied by the "wick-in-needle" technique, revealed high IFP values of 28.4±8.2 mmHg (n=7) in H460 tumors, compared to 14±10 mmHg (n=9) in MCF7 tumors and 0-5 mmHg in the flank muscle (n=20).

GdDTPA Steady state extracellular concentration maps derived from the T1 maps (see methods) demonstrated heterogeneous distribution throughout the tumors (image1 A and B) with higher concentrations at the tumors' periphery. Profiles of this distribution from one side of the tumor to the opposite side (Figure 1C,D) showed in the tumors periphery extracellular concentration similar to that found in the blood (~1 mM), in accord with a low or null IFP. Fast decline of the extracellular concentrations characterized H460 tumors, reaching null concentrations in the inner parts (1C), in accord with the high measured IFP. The central regions of MCF7 tumors demonstrated an extracellular contrast agent concentration of ~0.5 mM (figure 1D). This concentration was lower then the blood but higher then H460 GdDTPA extracellular concentration, with accord to the difference in IFP measured by the "wick in needle" method. The distribution of GdDTPA in the interstitial space did not correlate with the cell and capillaries density determined from the histological stained sections, supporting the presence of interstitial hypertension. Overall the average capillary density was 1.1±0.4% (n=7) in the H460 tumors and 1.3±0.6 % (n=5) in the MCF7 tumors.

Conclusion

The application of slow GdDTPA infusion and high resolution T1 measurements enabled us to map GdDTPA steady state concentrations in the interstitial volume of tumors. This distribution reflected the interstitial pressure distribution in agreement with the measurements performed by the "wick in needle" method. The capability of this contrast enhanced method to non-invasively estimate IFP distribution adds a new tool to detect barriers to drug delivery and improve management of chemotherapy. Furthermore it can help test new agents that reduce tumor IFP and consequently improve drug delivery.

References

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