Monitoring non invasively by Contrast enhanced MRI the decrease in tumors interstitial fluid pressure following collagenase treatment

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Many solid tumors show an increased interstitial fluid pressure (IFP), which forms a barrier to drug delivery. Efforts are currently directed towards identifying pharmacological agents that can decrease IFP and improve drug delivery into tumors. Herein we present a combined contrast enhanced MRI method that integrates the pharmacokinetic of GdDTPA during its slow i.v. infusion with the distribution of the contrast agent in tumors at steady state infusion. We show that this method can monitor collagenase induced decrease in IFP and the concomitant improvement in contrast agent delivery into H460 tumors.

Methods

The experiments were performed on Human H460 non small cell lung carcinoma cells inoculated subcutaneously in the flank 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.

Collagenase (Sigma-Aldrich, St Louis, MO, USA) treatment was applied by i.v. injection into the tail vein of 0.4 mg/kg body-weight of this enzyme diluted in PBS to 0.1% w/v (1 mg = 161 collagen digestion units).H&E staining were applied for morphological characterization of the tumors after collagenase treatment.

IFP was measured in tumors before and after collagenase treatment using the 'wick-in-needle' method as previously described (1).

The MRI images were acquired with a 4.7 T Biospec spectrometer (Bruker) 24 h before collagenase as well as ~2 h after the tratment. The MRI protocol included T1 measurements with fast SNAP inversion recovery (IR) sequence applying a non selective inversion pulse, inversion times ranging from 50 ms to 10 s, as well as fast low angle 3D GE acquisition with TE/TR = 2.1/18.3 ms and a 45^0 flip angle, matrix size 256x256, and a FOV of 4 cm.

GdDTPA was administered by slow infusion through the tail vein at a rate of 0.67 mmol/ kg/h for 2 hours. At 20 min of infusion GdDTPA reached steady state concentrations in the plasma. Following blood steady state, the other tissues also reached steady state GdDTPA concentration (1).

The 3D GE measurements were performed during the infusion. Images were analyzed using equations that include terms for the extravasation of the contrast agent from the capillaries and for the interstitial outward convection, as well as for the changes in plasma concentration during the infusion. The analysis yielded parametric maps of concentration gradient dependent transcapillary transfer constants (k^{trans}), extracellular volume fractions (ν_e), intravascular volume fractions (ν_p), and pressure gradient dependent transfer constants ($\pm k^{\Delta p}$) where + values indicate extravasation from the capillaries to the interstitium and – values indicate interstitial convection from the center of the tumor to the periphery.

T1 relaxation measurements were performed before infusion and at 90 min of infusion when GdDTPA concentration in the blood and tissues achieved a steady state. Maps of tissue GdDTPA concentrations at steady state were calculated from the T1 measurements (1). Maps of interstitial GdDTPA concentrations at steady state were calculated by dividing the tissue GdDTPA concentration in each voxel by the corresponding ν_e value calculated from the dynamic experiment described above.

Results

The 'wick in needle' measurements showed that IFP level of the H460 tumors decreased from 26.1 ±3.3 mmHg 24 hours before collagenase administration to 13.7±3.0 mmHg 5 h after the treatment (n=8, p=0.016- paired t test). Histological evaluation of hematoxylin-eosin stained slides of the tumors (n=12), removed 5 hours after administration of collagenase, showed predominantly densely cellular and disorganized sheets of viable pleomorphic cells as was observed in control tumors (n=12).

Slow infusion dynamic contrast enhanced MRI studies were performed in twelve ectopic H460 human tumors implanted in nude mice. The protocol was applied 24 h before collagenase administration as well as 3 h after the treatment. Pixel by pixel analysis of the images, as described above, yielded parametric maps of k^{trans}, $\pm k^{\Delta p}$, ν_e, and ν_p for each tumor. A paired t test in order to comparing the vascular parameters calculated before and after collagenase treatment showed that $k^{\Delta p}$ values inside the tumor, which inversely relate to IFP, increased significantly after the treatment while all the other pharmacokinetic parameters did not change significantly (Table 1).

Table 1: Statistical analysis of the vascular parameters in H460 tumors (n=12)

parameter	Before collagenase	After collagenase	p value
$K^{\Delta p}*10^{-3}$	-2.6±3.7	8.6±8.9	**0.016**
$K^{trans}*10^{-3}$	3.9±3.0	3.7 ±3.4	0.76
ν_e	0.23 ±0.07	0.24 ±0.06	0.64
ν_p	0.063 ±0.019	0.053 ± 0.027	0.19

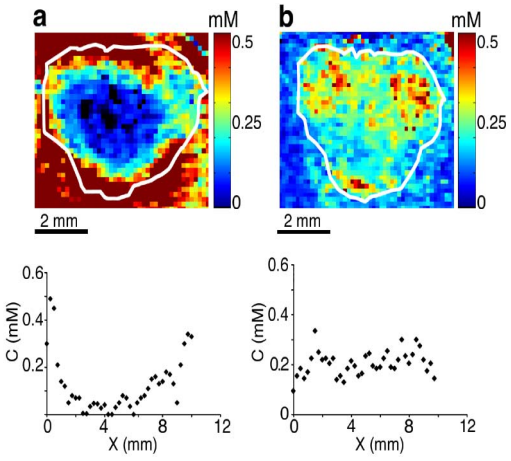

Figure 1: Collagenase induced changes in the steady-state tissue GdDTPA concentration of a H460 tumor.
a. Map and profile of the steady state tissue GdDTPA concentration 24 hours before the administration of collagenase (0.4 mg/kg body w)
b. map and profile of the steady state tissue GdDTPA concentration 5 hours after the i.v. administration of collagenase to the tumor in a

The T1 relaxation rate measurements confirmed the reduction in IFP, demonstrating an increase in the contrast agent tissue concentration after collagenase treatment (Figure 1). Interestingly, the concentrations in the tumor's periphery decreased after collagenase, most likely due to a decrease in the outward convection. Overall we found that GdDTPA interstitial concentration at steady state in the inner part of the tumors demonstrated a significant increase from 0.6±0.4 mM before to 0.8 ±0.5 mM after collagenase treatment (p=0.029 – paired t test).

Conclusion

We have demonstrated the application of an integrated CE-MRI method to test the efficacy of collagenase in reducing IFP and improving delivery into tumors. The decrease in IFP was reflected by increased pressure dependent transfer rate constants and increased interstitial steady state GdDTPA concentration in the central parts of the tumors. As many antiangioenic drugs work by a mechanism based on normalization of the capillary function and reduction in tumor IFP (2), This method may provide a powerful tool for testing the mechanism and efficacy of these drugs.

References

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