Hemodynamic Response Imaging for the Assessment of Anti-Angiogenic Treatment Response

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Background & Aims Tumor response to therapy is usually assessed by measurements of tumor size using morphological imaging techniques. However, tumor shrinkage can be observed only after weeks or even months, or even, may not occur at all, despite a positive response. Thus, the ability to detect early effects of tumor therapeutic response could facilitate decisions regarding therapy continuation or replacement. Novel approaches directed to the complex interactions between a tumor and its microenvironment in the angiogenic process will hopefully strengthen the therapeutic armamentarium against cancer. Since anti-angiogenic therapy may not lead to substantial tumor mass reduction, conventional tumor size measurements may be insensitive. Therefore, identification of new noninvasive monitoring techniques for assessing tumor response is a major necessity in this field. Recently, we demonstrated the feasibility of *Hemodynamic Response Imaging (HRI),* an fMRI method combined with hypercapnia and hyperoxia for monitoring changes in liver perfusion and hemodynamics[1,2]. In the present study, we aimed to improve therapeutic response assessment by evaluating the therapeutic effects of a novel anti-angiogenic therapy on colorectal liver metastases (CRLM) by *HRI*. This method allowed us to obtain functional parameters that complement the anatomical information.

Methods ***Animals***: CB6F1 mice underwent splenic injection with CT-26 colon cancer cells to generate liver metastases. Mice were treated by daily i.p. injections of "*Hamsa*", a novel treatment based on the combination of low-dose cytotoxic agent, COX1 inhibitor, a histamine type 2 (H2) receptor antagonist and hypoxia-like inducing agent, or inert vehicle alone. Treatment was started on the day of tumor appearance in T_2W images (day 15±2). Tumor progression was monitored by MRI twice a week. Animals were sacrificed at the end of the experiment and their livers were harvested for histology. ***MRI:*** Experiments were performed on a 4.7T Bruker Biospec spectrometer using a 3.5 cm bird cage coil. Hepatic volumetric assessment was acquired by serial coronal and axial T_1W SE images (TR/TE=250/18ms). Tumor assessment was done using T_2W fast SE images (TR/TE=2000/40ms). Changes in hepatic hemodynamics were evaluated from T_2*W GE (TR/TE=147/10ms) images acquired during breathing of air, air-CO_2 (5% CO_2), and carbogen (95% oxygen; 5% CO_2) as described[1]. Data analysis was performed using a home written IDL software. CO_2 and O_2 reactivity maps are given as the percentage of change of signal intensity (ΔS)[1].

Results

Previously, we demonstrated the feasibility of *HRI* for monitoring changes in liver perfusion during CRLM development[3]. In this study, we assessed the hepatic perfusion changes that occur during *Hamsa* therapy, by using *HRI*. Morphological estimation of tumor growth kinetics showed that *Hamsa* treatment delayed tumor progression for a period of 19 days in average. Subsequently, however, the metastases started to grow and additional CRLM developed (Fig.1). Moreover, *HRI* maps demonstrated that until the time of tumors' outburst, *HRI* map values were similar to those of the control-treated mice (Fig.2A,B, Fig.3). While later, there was a significant decrease in vessel reactivity to the gases and the lesion borders became blurred, resulting in uncharacteristic *HRI* maps (Fig.2C, Fig.3). Indeed, histological analysis confirmed the *HRI* results, revealing undefined tumor borders and a change in tumor growth characteristics (replacement mode) in the *Hamsa*-treated mice (Fig. 4B). Since vessel reactivity in the entire liver was attenuated, we treated additional control mice with *Hamsa* (without CRLM) and analyzed their hemodynamic profile. Indeed, we also found alterations in the *HRI* maps in these mice (Fig. 2D), that were further confirmed by the appearance of small necrotic foci in the histological slides and alterations in the liver vasculature as revealed by CD31 immunostaining.

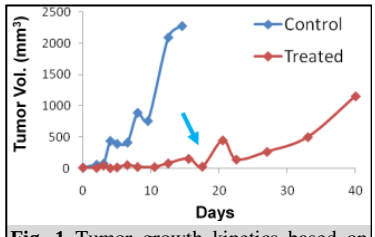

Fig. 1 Tumor growth kinetics based on T_2W fast SE images. Control (n=14) vs. *Hamsa* treated (n=8).

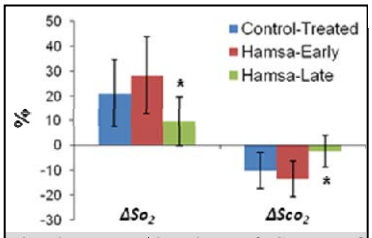

Fig. 3 Mean ΔS values of CRLM of control-treated (n=26) vs. early and late treatment stages (n=13, *p<0.005)

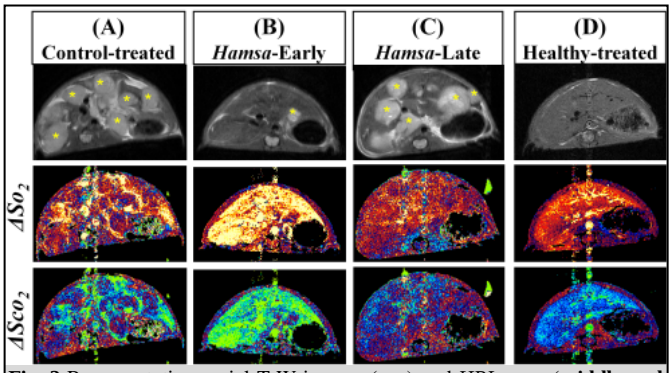

Fig. 2 Representatives axial T_2W images (**top**) and *HRI* maps (**middle and bottom**) of control-treated (A), early *Hamsa* treatment (B), late *Hamsa* treatment (C) and healthy- *Hamsa* treated (D) mice. (CRLM marked by yellow asterisks; ΔS maps scale bar -30% +30%)

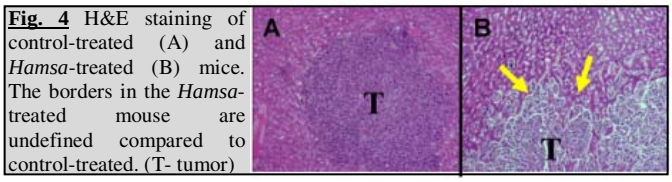

Fig. 4 H&E staining of control-treated (A) and *Hamsa*-treated (B) mice. The borders in the *Hamsa*-treated mouse are undefined compared to control-treated. (T- tumor)

Conclusions

In this study, we have shown that "*Hamsa*" treatment reduced tumor growth and thus prolonged mice survival. However, it induced a change in the CRLM growth morphology which was reflected in *HRI* maps. Thus, *HRI* utilization offers a new noninvasive method for monitoring anti-angiogenic therapy response and may facilitate detection of tumor deterioration.

References: *[1]Barash,H;Gross,E;Matot,I;Edrei,Y;Tsarfaty,G;Spira,G;Vlodavsky,I;Galun,E;Abramovitch,R;Radiology 243(3),2007; [2]Barash,H;Gross,E; Edrei,Y; Spira,G;Vlodavsky,I;Galun,E;Matot,I;Abramovitch,R;Hepatology; [3]Edrei,Y;Galun,E;Gross,E;Pikarsky,E;Abramovitch,R; #1752, ISMRM[2006];*