Simultaneous examination of metabolism and perfusion of embolized hepatocellular carcinoma in rats by hyperpolarized fumarate and copolarization of pyruvate and urea

Stephan Düwel[1,2], Markus Durst[1,3], Concetta Gringeri[3], Yvonne Kosanke[4], Claudia Gross[4], Martin A Janich[3], Markus Schwaiger[5], Axel Haase[1], Steffen Glaser[2], Rolf F Schulte[3], Rickmer Braren[4], and Marion I Menzel[3]

[1]Institute of Medical Engineering, Technische Universität München, Garching, Germany, [2]Chemistry, Technische Universität München, Garching, Germany, [3]GE Global Research, Garching, Germany, [4]Institute of Radiology, Klinikum rechts der Isar, Technische Universität München, München, Germany, [5]Institute of Nuclear Medicine, Klinikum rechts der Isar, Technische Universität München, München, Germany

Introduction: Hyperpolarized 13C metabolic MR spectroscopic imaging of pyruvate and its down-stream metabolites allows real-time in-vivo studies of energy metabolism in healthy and tumor tissue [1]. Recently, the simultaneous and independent investigation of tumor perfusion was studied using metabolically inactive hyperpolarized agents as a direct signal source that is background-free [2]. Perfusion is an important parameter in tumors, where the production of malate from hyperpolarized fumarate can be used to assess cell necrosis [3]. In this study, we compared the perfusion information given by hyperpolarized [1,4-$^{13}C_2$]fumarate, [1-^{13}C]pyruvate and [^{13}C,$^{15}N_2$]urea in a group of five rats exhibiting hepatocellular carcinoma (HCC) before and after transcatheter arterial embolization (TAE).

Methods: Unifocal orthotopic tumor transplants were generated by injection of 4x10^6 syngeneic McA-RH7777 rat HCC cells into the right lateral liver lobe of five Buffalo rats; TAE was performed by administering 1ml of 50% EmboCept® through the gastroduodenal artery, so that the embolizing agent penetrates into small tumor vessels and produces a transient vessel occlusion [4]. [1,4-$^{13}C_2$]fumaric acid was hyperpolarized using a HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK) at a microwave frequency of 94.115 GHz, dissolved in 40mM phosphate buffer in D_20, NaCl and EDTA for physiological pH and temperature, producing 20mM hyperpolarized fumarate. [1-^{13}C]pyruvic acid and [^{13}C,$^{15}N_2$]urea were hyperpolarized in frozen layers at a microwave frequency of 94.105 GHz, dissolved in 80mM Tris Buffer in D_2O, producing 80mM hyperpolarized pyruvic acid. Both solutions were injected with a dose of 5ml/kg at a rate of 0.2ml/s into the tail vein of the rats. For anatomical reference, T_2-weighted, liquid suppressed fast spin echo ^{1}H images were acquired. All experiments were performed on a Signa HDxt 3.0T clinical scanner (GE Healthcare, Milwaukee, WI, USA). A ^{13}C-IDEAL spiral CSI imaging sequence was adapted (FA=10°, TR=500ms, nTE=7, dTE=1.12ms) to the expected substances and data reconstruction was performed according to [5] resulting in one complete ^{13}C metabolite image set every 4s. Similarity between images depicting different substances was evaluated using a publicly available algorithm quantifying structural similarity [6]. In addition, ROIs were manually drawn around the tumor and kidney. For each animal, for which full data sets were available (proton images in sufficient quality for clear identification of the tumor and hyperpolarized data sets for both pre and post TAE), the average signal intensity of the tumor with respect to the average signal intensity of the kidney was calculated.

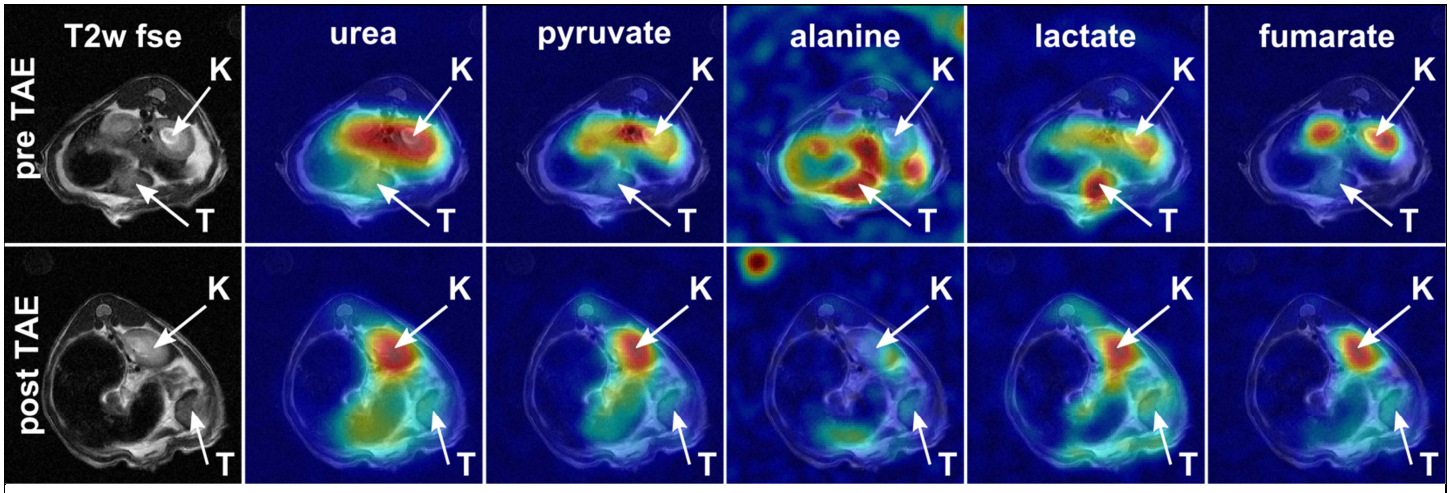

Fig 1: Representative axial slice through HCC rat tumor before (first row) and after (second row) TAE. The anatomical location of the kidney (K) and the tumor (T) are shown; the tumor's position changed during embolization. False-color ^{13}C IDEAL spiral CSI images are superimposed on the ^{1}H image to show the spatial distribution of the hyperpolarized substances. The color scale indicates the relative signal intensity compared to the maximum intensity within each image.

Results: Animal subjects were examined 12 to 13 days after cell implantation with the same protocol one day before and one day after embolization. Twenty seconds after the first injection, an IDEAL spiral CSI image of hyperpolarized fumarate and malate (not shown) was taken. About one hour later, right after the second injection, the images of copolarized urea and pyruvate as well as its metabolic products were acquired. Anatomical reference images were taken in between the two injections. Before embolization (fig. 1, first row), urea, pyruvate and fumarate light up in similar places; the same is true for the spectroscopic images taken after the embolization (fig. 1, second row). The structural similarity index (fig. 2) quantitatively confirms this observation, resulting in values of 0.80±0.04 and 0.78±0.05 for pyruvate and fumarate, respectively (n=10). We found that urea, pyruvate and fumarate signals behaved similarly, showing an average increase from pre TAE to post TAE of tumor to kidney signal by a factor of 1.4±0.3, 1.3±0.1 and 2.6±0.6, respectively. In contrast, the alanine and lactate tumor to kidney signals on average decreased by a factor of 0.8±0.2 and 0.7±0.2 respectively (n=3).

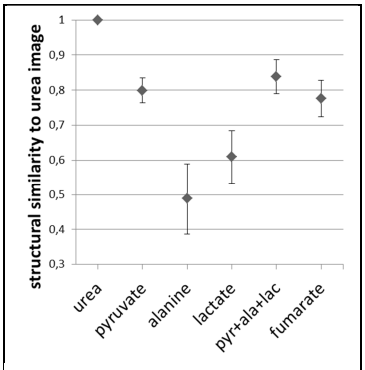

Fig 2: Structural similarity index (SSIM) with respect to urea for all substances and a selected sum. Mean values and standard deviations are calculated from five animals, resulting in n=10 data sets of five images each.

Conclusion and Discussion: This study is to our knowledge the first to simultaneously investigate metabolism and perfusion of an embolized rat by using hyperpolarization methods. We have shown that hyperpolarized urea, pyruvate and fumarate all show consistent information on tumor perfusion with respect to kidney perfusion. On the one hand, this finding can be used to independently confirm perfusion data by these three substances. On the other hand, perfusion information could be based solely on pyruvate or fumarate injections without the need for a copolarization with urea. Subsequent perfusion imaging by Gd-DTPA DCE-MRI will be used for further validation and findings will have to be confirmed by histological analyses of tumor tissue. Future work with higher spectral resolution could combine fumarate and pyruvate into one copolarization [7] without having to add urea as a third component. While reducing the complexity of the copolarization process, this could still provide information about perfusion, metabolic activity and necrosis simultaneously.

References: [1] Brindle KM et al. MRM 66 (2011) 505 [2] von Morze C et al. MRI 30 (2012) 305 [3] Gallagher FA et al. PNAS 106 (2009) 19801 [4] Braren R et al. J Hepatol 55 (2011) 1034 [5] Wiesinger F et al. MRM 68 (2012) 8 [6] Wang Z et al. IEEETIP 13(2004) 600 [7] Wilson DM et al. JMR 205 (2010) 141.

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