Comparison of Diffusion Weighted Imaging with [^{18}F]-FLT Uptake in a Human Colon Cancer Xenograft Model using Treatment Strategies

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Introduction:
Diffusion-weighted magnetic resonance imaging (DWI) enables the in vivo characterization of biological tissues on the basis of their water diffusion properties, hence can be a measure of tissue cellularity and therefore is a potentially useful diagnostic tool for monitoring cancer therapy. An emerging topic in the field of oncological MRI is, whether the information obtained by DWI measurements is complimentary to or even a substitute for Positron-Emission-Tomography (PET) methods. However the majority of these studies focused on comparisons of ADC measures with [^{18}F]FDG-PET-Tracers that is primarily a marker for glucose metabolism. Therefore we focused in this study on PET imaging with [^{18}F]FLT, which provides information on cell proliferation – a measure more closely related to tissue cellularity. We investigated tumor proliferation, functional status and growth of a human colon adenocarcinoma cell line (HCT116) in female SWISS nude mice by [^{18}F]FLT-PET and apparent diffusion coefficient (ADC) MRI using the cytostatic drug docetaxel as treatment over a period of eight days.

Materials and Methods:
Comparison between PET and DWI was performed on a small animal PET scanner and a 7 T small animal MRI system, using female SWISS nude mice (n=12), average weight ca. 20 g. Animals were anesthetized using 1.5% Isoflurane in oxygen, and placed on a MRI mouse bed. HCT116 cells were cultured over three weeks (passage 7-10) before injection. After subcutaneous inoculation of 10^7 HCT116 cells into the upper right flank of the mice a visible tumor appeared after ten days and baseline imaging with PET and MRI measurements was performed. Every second day the weight of the animals was measured as well as the tumor size using callipers. After baseline imaging, docetaxel was injected intravenously the following day (first day of treatment) at a concentration of 15 mg/kg. No additional injection was performed. Docetaxel was used in its clinical available form (4 mg/mL in NaCl). Further PET and MRI imaging was performed on day three, six and eight after baseline imaging. After the last PET and MRI measurements, blood, background tissues and tumor were taken for gamma counting. The tumor was fixed in paraffin for immunohistochemical staining. For the dynamic and static PET scans mice were administered on average with 13 MBq [^{18}F]FLT. The uptake times for the static PET scans were 90min for [^{18}F]FLT. Following the PET scans, each mouse was transferred on the same bed to the MRI scanner. DWI was performed in sagittal direction (b = 150, 250, 400, 600, 800, 1000 sec/mm^2, TE = 112ms, TR = 5000ms, Δ = 41 ms, δ = 20 ms). ADC maps were calculated and coregistration of the MRI and PET images was performed. For the quantitative analysis of tumor tracer uptake, regions of interest (ROIs) were drawn manually on sagittal images.

Results:
On the baseline imaging day, mean tumor size for both control and docetaxel treated groups was 0.29 ± 0.15 cm^3. Tumors in the control group grew throughout the study, with a mean size of 1.10 ± 0.37 cm^3 on day 8. In comparison, tumors in the docetaxel treated group showed growth arrest, with a mean size of 0.40 ± 0.22 cm^3 on day 8. The PET measurements showed a slightly increased tumor uptake of [^{18}F]FLT in the control group over the measurement period of eight days (from 4.73 ± 0.61 %ID/cc on the baseline measurement to 5.09 ± 2.02 %ID/cc on day 8). In contrast, the docetaxel treatment group showed significantly (p=0.02) increased [^{18}F]FLT uptake (from 4.68 ± 0.60 %ID/cc on the baseline measurement to 6.07 ± 0.36 %ID/cc on day 8).
The diffusion weighted imaging revealed a mean baseline ADC value of 0.69*10^{-3} ± 0.10 mm^2/sec with the control group showing no significant change in ADC values over the period of the study. The ADC values of the docetaxel treatment group showed highly interesting results. The ADC values increased after administration of the cytostatic drug and remained at the same level for the following measurement days (from 0.70*10^{-3} ± 0.09 mm^2/sec on the baseline measurement day to 0.84*10^{-3} ± 0.02 mm^2/sec on day 3 and 0.84*10^{-3} ± 0.10 mm^2/sec on day 8). Focusing on the tumor regions showing no [^{18}F]FLT uptake during this study, the increase of the ADC values from the baseline day to day 3 was significantly high (p=0.005) compared to the ADC values of the whole tumor (from 0.67*10^{-3} ± 0.04 mm^2/sec on the baseline measurement day to 0.89*10^{-3} ± 0.03 ^{3} mm^2/sec on day 3). The co-registration of [^{18}F]FLT images with the corresponding ADC maps revealed an inverse spatial correlation between PET tracer uptake and ADC values for all measurements, particularly in highly heterogeneous or necrotic tumor regions (Fig.1).

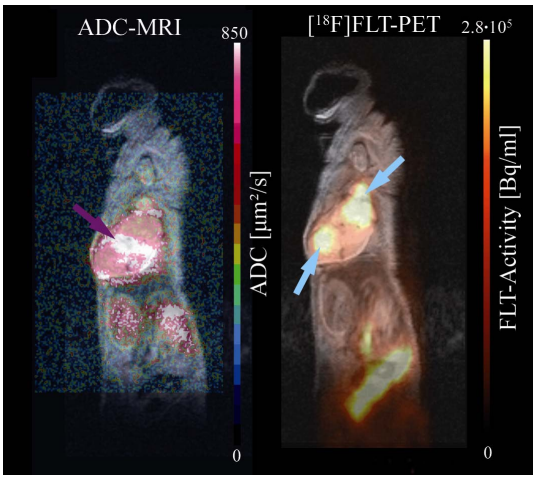

Figure 1: Sagittal section of a subcutaneous HCT116 tumor in a mouse. Corresponding ADC-MRI and FLT-PET images clearly reveal an inverse correlation between diffusivity and highly proliferating regions.

Discussion and Conclusions:
Diffusion-weighted magnetic resonance imaging (DWI) in combination with PET techniques is a powerful tool to monitor cancer therapy. PET imaging with [^{18}F]FLT provides information on cell proliferation and the apparent diffusion coefficient enables the observation of the water diffusion in a solid tumor. Here, we show the correlation between the [^{18}F]FLT PET imaging and ADC in a HCT116 colon cancer mouse model. The control group showed no significant changes of the ADC values over a period of eight days whereas the [^{18}F]FLT was slightly increased. A second group of animals were treated with the cytostatic drug docetaxel on the first day after the baseline PET and MRI measurements. This group showed a significantly (p=0.02) increased [^{18}F]FLT uptake 24 hours after the administration of the drug. In contrast the tumor showed growth arrest over a period of eight days. Some chemotherapeutic agents that block the thymidine synthesis have been shown to cause a temporary activation of the thymidine kinase activity and thus an increased [^{18}F]FLT uptake[1]. Also the ADC values increased significantly after administration of the cytostatic drug, but stayed on the same level on the following measurement days. Docetaxel (taxan drug class) treatment causes the promotion of microtubule polymerisation leading to cell cycle arrest that could explain the increased [^{18}F]FLT uptake as well. It also causes apoptosis and cytotoxicity which could lead to an increased diffusion and ADC[2].
ADC maps revealed an inverse spatial correlation to the [^{18}F]FLT uptake demonstrating the relationship between water diffusion in necrotic regions and the thymidine kinase activity in proliferating cells (Fig.1). Such complementarities have been already shown for the glucose analogon [^{18}F]FDG but not for the specific cell proliferation marker [^{18}F]FLT. The inverse correlation between MR diffusivity and [^{18}F]FLT-PET therefore opens new insights for the usability of ADC measurements as a diagnostic tool in oncology.

References:
[1] Haubner R, PET radiopharmaceuticals in radiation treatment planning – Synthesis and biological characteristics, Radiotherapy and Oncology, 96 (2010), 280-287.
[2] Clarke SJ and Rivory LP, Clinical Pharmacocinetics of Docetaxel, Clin. Pharmacokinet. 36 (1999), 99-114.