Characterization of tumor microvascular structure and permeability by MRI and intravital confocal imaging

N. K. Reitan[1], M. Thuen[2], and P. E. Goa[3]
[1]Department of Physics, NTNU, Trondheim, Norway, [2]Department of Circulation and Medical Imaging, NTNU, Trondheim, Norway, [3]Department of Radiology, St. Olavs Hospital, Trondheim, Norway

Introduction

Solid tumors are characterized by abnormal organization, structure, and function of blood vessels.[1,2] These structural abnormalities give rise to enhanced vascular permeability and may predict therapeutic response. Tracer kinetic analysis of contrast-enhanced MR images allows estimation of physiological parameters such as blood volume, blood flow and vessel permeability. However, the heterogeneous tumor microvasculature induces uncertainty in quantification of parameters, which may be overcome by validation of results by another imaging technique. Intravital optical microscopy using confocal laser scanning or two-photon microscopy has superior spatial resolution compared to MRI, and the heterogeneous distribution of several microvascular characteristics can be imaged simultaneously and colocalized in three dimensions.

Materials and Methods

By using confocal laser scanning microscopy (CLSM) and MRI on a 7T scanner we studied microvascular architecture and permeability in human osteosarcoma tumors growing in MR-compatible dorsal window chambers in mice (BALB/c nu/nu). *Microvascular structure:* For imaging of microvascular structure, intravascular tracers were injected. FITC(fluorescein isothiocyanate)-dextran (2 MDa) was used for CLSM (Fig. 1a) and Albumin-(Gd-DTPA) for MRI (Fig. 1b) using a T1-weighted 3D Flash sequence (TR 15 ms, TE 2.4 ms). Structural parameters that indicate the complexity of the vascular architecture were estimated from the CLSM images. Fractal dimension describes irregular, tortuous contours and branching structures which are characteristic for tumor microvasculature. Fork density is an estimate of the number of neo-formed vessels and was defined as the number of vessel nodes in the image. Vascular density was calculated as the fraction of the image area covered by vessels. *Permeability:* TMR(tetramethylrhodamine)-dextran (40 kDa) and Gadomer was used as molecular tracers for dynamic imaging by CLSM and DCE-MRI, respectively. The fluorescence intensity as a function of time was measured by CLSM in the intra- and extravascular space (Fig. 2a-c). The extravasation rate K_i was calculated as the terminal slope of the curve in a Patlak plot (Fig. 3a).[3] Dynamic contrast enhanced MRI (DCE-MRI) was aquired (Fig. 2d-f) using a T1-weigthed spin echo Rare sequence (TR 200 ms, TE 7 ms, 350 repetitions, time resolution 6.4 s) and permeability indicators were estimated from the contrast agent concentration-time curve (Fig. 3b). The initial slope and the area under the curve (*AUC*) for 10 min post injection of Gadomer were directly extracted from the experimental data, and the volume transfer constant K^{trans} was calculated by fitting to Tofts model.[4]

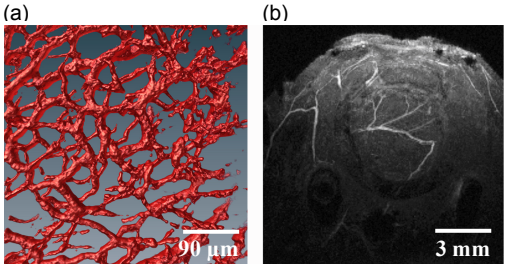

Figure 1. Vasculature in tumor tissue in a dorsal window chamber imaged by CLSM (a) and MRI (b) showing the difference in resolution for the two techniques

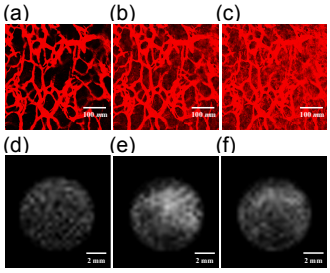

Figure 2. Dynamic time series of tumor in a dorsal window chamber, acquired by CLSM (a: 2min, b: 9min, c: 25min post-contrast) and DCE-MRI (d: pre-contrast, e: 5min and f: 37min post-contrast).

Results and Discussion

Because of the rapidly expanding, chaotic, and branched nature of angiogenic vessels, fractal dimension, vascular density, and fork density assumed higher values for the abnormal tumor vasculature than for normal vessels. A high positive correlation was found between these structural parameters, which have previously been reported to yield information about vascular architecture, tumor growth and angiogenesis.[5] Furthermore, a positive correlation was found between permeability and microvascular structure. The vascular endothelial growth factor (VEGF-A), which is responsible for angiogenesis, is also a strongly permeabilizing agent, and neoformed angiogenic microvessels are therefore generally leaky..A correlation was also found between permeability indicators obtained by CLSM and DCE-MRI (Fig. 4). However, due to a higher spatial resolution, CLSM revealed a high degree of heterogeneity in the tissue for both vascular architecture and permeability.

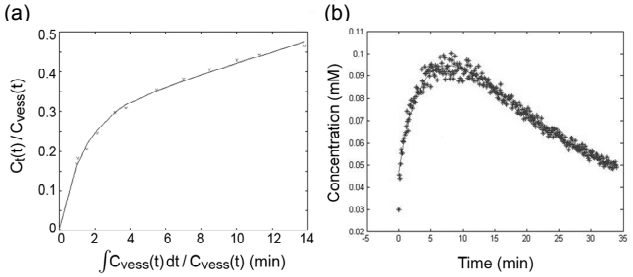

Figure 3. Dynamic measurements of tumor permeability. (a) Patlak-plot from CLSM fluorescence intensity-time series. (b) Tofts model fitted to DCE-MRI curve showing concentration versus time after injection of Gadomer.

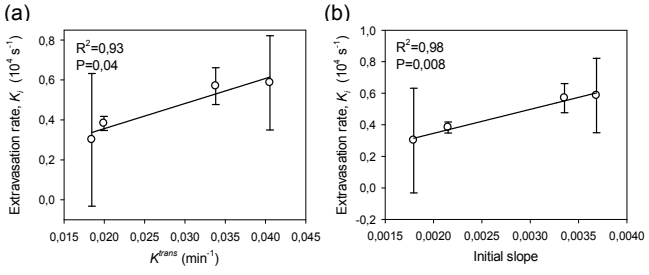

Figure 4. Correlation between the extravasation rate K_i obtained by CLSM and (a) K^{trans} and (b) the initial slope of the DCE-MRI concentration-time curve for tumor tissue in a dorsal window chamber.

Conclusion

This study demonstrates that the dorsal window tumor model gives a unique opportunity to use CLSM and MRI as supplementary and complementary techniques. CLSM can both validate results obtained with MRI and provide insight into the spatial heterogeneous microenvironment on a microscopic level that is not accessible with MRI.

References

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