"Clustering and rationing" of Gd-enhanced voxels by injection of Gd-labelled RBC, a tool to quantify functional information from MR images

Enza Di Gregorio[1], Giuseppe Ferrauto[1], Stefania Lanzardo[1], Eliana Gianolio[1], and Silvio Aime[1]
[1]*Molecular Biotechnologies & Health Sciences, University of Torino, Torino, Italy*

Introduction: In the last two decades, many MRI contrast agents (CAs) have been suggested as blood pool CAs and some of them are currently used in clinical and/or pre-clinical applications. One of the issues related to the use of these agents deals with their extravasations. One way to get rid of this problem is to use Gd-labelled RBCs that are 100% confined in the blood vasculature. The possibility of internalizing molecules inside red blood cells by applying osmotic shock has already been exploited and herein this technique is efficiently applied to label RBCs with Gd-HPDO3A (ProHance, Bracco Imaging Research.). In this contribution it is reported that Gd-loaded erythrocytes can be efficiently used as blood pool contrast agents suitable for the evaluation of vascular volume in subcutaneous tumors. Results obtained by using Gd-loaded RBCs are compared with the ones obtained by using ProHance or a Gd-complex that efficiently bind Serum Albumin. On this basis contrast-enhanced MR images have been analyzed as follows: i) selection and clustering of Gd-enhanced pixels for the different agents and ii) rationing the selected pixels for the same ROI to access a quantitative information on vascularity and permeability. **Methods:** Murine RBCs have been isolated by centrifugation. The loading of Gd-HPDO3A has been obtained by applying hypotonic methodology. Briefly, erythrocytes were placed for 30 min at 4°C into a hypotonic solution (160mOsm/l) containing the paramagnetic agent to be loaded. The normal morphology of erythrocytes was then restored by returning the osmolarity of the solution to an isotonic condition (280mOsm/l) with the addition of a proper concentration of PBS. After this treatment the samples were extensively washed to eliminate the not internalized molecules. Mouse tumour models were prepared by the subcutaneous injection of TSA murine breast cancer in Balb/C mice. Gd-labelled RBCs were successively injected into mouse tail vein and T_{1w} images were acquired at 7T on a Bruker Avance 300MHz spectrometer. The enhancement of contrast has been evaluated voxel by voxel and maps of vascularization/perfusion have been obtained. **Results:** Three types of distribution maps have been acquired by using the following different contrast systems*:* i) Gd-HPDO3A, ii) Serum Albumin (SA)- binding Gd-complex and iii)Gd-HPDO3A-labelled RBCs. For Gd-HPDO3A, the entire rim region appears markedly hyperintense as, well as several, large inner areas (Fig.1A). These small sized agents escapes massively from the vessels, in the tumor region where the permeability of endothelium is high, and therefore it cannot be used to establish the vascular volume. The Albumin-binding agent appears to yield similar results although the larger size of Albumin-Gd-complex adduct is limiting its perfusion ability in the inner part of the tumor (fig.1B). Conversely, by using Gd-HPDO3A-labelled RBCs only limited inner areas and the rim appear hyperintense, with intensity values lower than the ones shown by the other two compounds (Fig.1C). A more detailed and quantitative analysis has been carried out by clustering the voxels. In practice, the voxels of the ROI (covering the tumor region) are clustered according to the observed signal enhancement (Fig.1D). By looking at the number of voxels enhanced by the Albumin-binding CA or Gd-HPDO3A, these two CAs do not appear to represent good reporters of the vessel distribution as the characteristic permeability of the tumor vessels yields to their large spread-out over the entire tumor region. Instead, upon injection of Gd-RBCs, the voxels that display a detectable SI% enhancement cover only *ca*. the 60% of the tumor ROI and more in detail the majority of the voxels have a SI%<30%. On the basis of these results Gd-loaded RBCs have been used to follow the increase of vascular volume during tumor development. **Conclusions:** Tumor vasculature is probably one of the most intensively investigated aspects of the tumor microenvironment since tumors need to develop its own blood vascular network to obtain enough amounts of nutrients and oxygen. For this reason the evaluation of vascular volume inside tumor region is of huge interest for the tumor staging and for the evaluation of anti cancer treatments. In the herein reported work it is shown that, upon loading with Gd-based contrast agents, erythrocytes can act as excellent blood pool MRI contrast agents and by the application of a ratiometric approach is it possible to access to quantitative information on the vascularity level in the tumor region. **References:** Bell LK, Ainsworth NL, Lee SH, Griffiths JR. NMR Biomed, 2011;24(6):612-35.

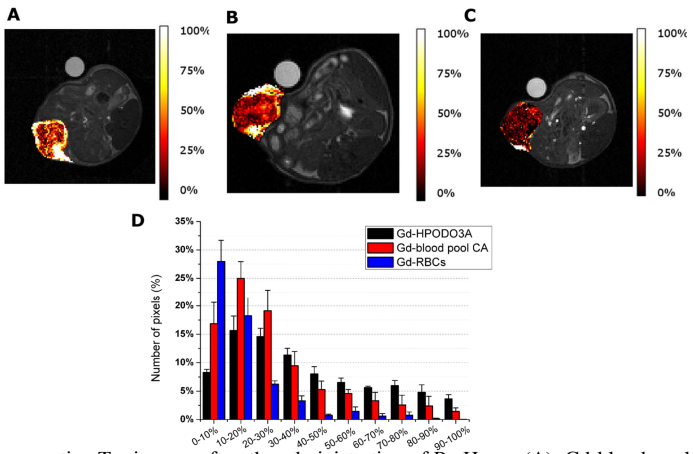

Fig.1 Representative vascular density maps in tumor region overlapped on the respective T_{1w} images after the administration of ProHance (A), Gd-blood pool CA (B) or Gd-RBCs (C); (D) Vascular density voxel distribution in tumor region after the administration of the three described CAs. The mean of three independent experiments carried out on three mice at the same stage of tumor growth is reported.