Molecular MRI and DCE-US to evaluate anti-angiogenic therapies in kidney tumor xenografts

Ludovic de Rochefort[1], Ingrid Leguerney[1], Marie Poirier-Quinot[1], Sandra Robin[1], Xavier Violas[2], Rose-Marie Dubuisson[1], Stéphanie Pitre-Champagnat[1], Luc Darrasse[1], Philippe Robert[2], and Nathalie Lassau[1]

[1]*IR4M, Univ. Paris-Sud, CNRS, UMR 8081, Orsay, France,* [2]*Experimental Imaging, MRI unit, Research Division, Guerbet, Aulnay-sous-bois, France*

PURPOSE

In the future, molecular imaging is expected to enhance disease diagnosis and monitoring [1]. Molecular MRI requires sensitivity, specificity and quantification to probe molecular markers in the low concentration range. Functionalized superparamagnetic iron oxides nano-objects are potential candidates filling these three criteria [2]. Here, molecular MRI with a functionalized iron oxide nanoemulsion and DCE-US are applied to evaluate the early response to three anti-angiogenic drugs in xenografted kidney cancer in mice.

MATERIAL AND METHODS

Animal model and treatments: 24 nude mice were xenografted (3.10^6 A498 cells). After 3 weeks, they had a first imaging session at baseline (D0) comprising US and MRI. They were then separated in 4 groups: 1 control (saline) and 3 treated with anti-angiogenic drugs: VEGF inhibitor (monoclonal anti-body, bevacizumab, 5mg/kg), thyrosine kinase inhibitor (sunitinib, 40mg/kg) and m-Tor inhibitor (everolimus, 10mg/kg). They were imaged after 3 days of treatment (D3).

Imaging protocol: At D0 and D3, DCE-US first was done with Toshiba Aplio XV using a high frequency probe and microbubbles (Sonovue, Bracco). MR acquisitions (Fig.1) were then performed at 1.5 T (Philips Achieva), using a conventional 23mm-diameter surface coil. Mice were anesthetized with a mixture of isoflurane+O_2 and breathing patterns were monitored (SA Instruments, Inc, USA). After localization, T1W and T2W, a diffusion weighted sequence was applied to quantify apparent diffusion coefficient (ADC). It consisted in a multi-slice 2D spin-echo-EPI, TR/TE=1.9s/72ms, 1 mm thick slice, 0.5 mm in plane resolution, 2 diffusion gradients were used (b=0/600 s.mm^{-2}). T2* was mapped using a 3D gradient echo multi-echo sequence (TR/TE/dTE=90/6/9.7, 0.3x0.3x0.5 mm pixel size, 220Hz/pix, Tacq=4.2min). An USPIO-RGD based nanoemulsion binding αvβ3 (P04000 Guerbet, 100µmolFe/Kg) was injected in the tail vein. To estimate the first pass in the tumor, a dynamic single-slice 2D T2* mapping was applied during 1min20s (temporal resolution 1.8s). 3D T2* mapping was then performed repeatedly for 1 hour.

Data analysis: Tumor volume was estimated 1) from US based on the measurement of 3 orthogonal tumor lengths, 2) from MRI using manual ROI tracing in each slice encompassing tumor. Area under the curve (AUC) from DCE-US estimated blood volume. On a central slice, mean ADC was calculated from the mean signal decay over the tumor assuming exp(-b.ADC). The 1st pass clearance was estimated from the dynamic 2D scan: a mean R2* was estimated over the tumor, and its temporal evolution was fitted to a line. Finally, the mean R2* over the tumor from the repeated 3D scan was estimated and analyzed up to 1 hour post injection.

RESULTS

Tumor volumes tend to decrease with treatment using both US and MR estimations (Fig.2a). They were significantly different between control and bevacizumab group at D3 for MRI ($p<0.01$). AUC calculated with DCE-US at D3 was significantly lower for bevacizumab group ($p<0.01$) and not significant for the 2 other treatments (Fig.2b). ADC value at D0 was $1.12x10^{-3} \pm 0.14x10^{-3}$ mm^2.s^{-1} (mean±std). There is a trend for an increased ADC at D3 in controls, a lower increase for sunitinib, a stabilization for everolimus and a decrease for bevacizumab (Fig.2c). First pass R2* clearance at D0 was estimated at -1.6 ±1.8s^{-1}min^{-1} (mean±std) indicating a slow clearance after injection. At D3, clearance was drastically accelerated in the control group (Fig. 2d), and remained close to D0 value for everolimus group. R2* quantification at D3 1 hour post-injection was reduced from 23.1±4.6 s^{-1} in the control group to 7.9±4.8 s-1 ($p<0.01$) for everolimus, 13.5±7.9 s-1 ($p<0.05$) for bevacizumab and not significant for sunitinib. The evolution of dR2* at D0 (Fig.3) displays a slow clearance reaching a plateau after 1 hour. At D3, while the everolimus group had the same trend, a plateau is obtained rapidly for control, sunitinib and bevacizumab.

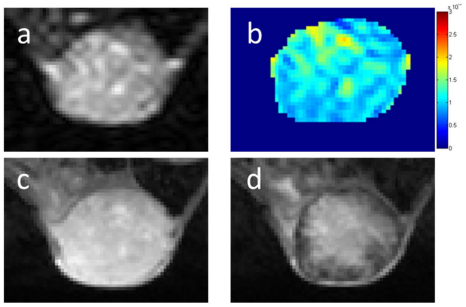

Fig. 1: Typical MR images obtained on tumor. (a) T2W image, (b) ADC map, (c) pre- and (d) 1-hour post-injection T2*W images.

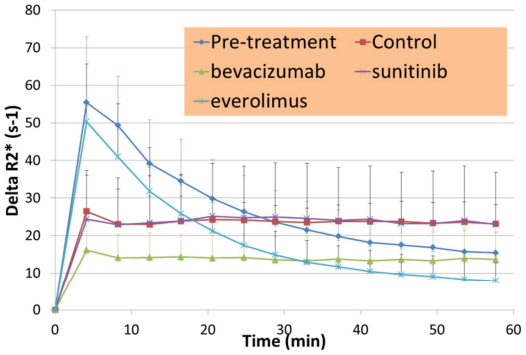

Fig. 3: Mean change in R2*as a function of time after injection before (dark blue) and after treatment (mean+SEM).

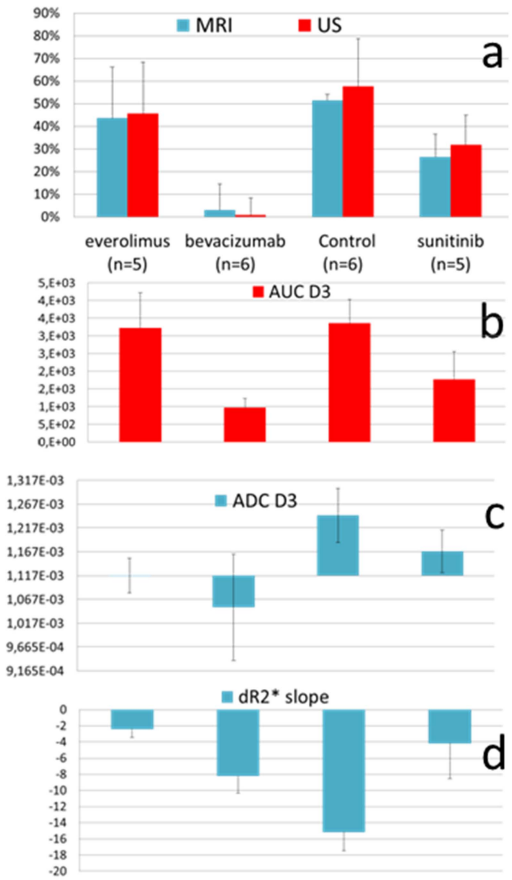

Fig. 2: a) Tumor growth evaluation in the 4 groups from US and MRI. b) AUC at D3 from DCE US (arbitrary units). c) Mean change in ADC at D3 in mm^{2}s^{-1}. Bars have been centered on the mean value before treatment. d) dR2 slope during the 1st minute injection at D3 (given in s^{-1}min^{-1}). (mean±standard error of mean)

DISCUSSION AND CONCLUSION

We presented a multimodal molecular MRI and DCE-US approach at clinical field B_0 to evaluate anti-angiogenic drug efficacy in xenografted kidney cancer in mice. Various differences between control and treated groups were observed in tumor volume, perfusion and ADC. Molecular imaging with αvβ3 targeting contrast agent is complementary to detect the effects of anti-angiogenic drugs with different mode of action. αvβ3 integrin expression evaluated 1 hours post injection after clearance tends to increase for control and sunitinib, and reduce for everolimus. The action of repeated injection had a drastic effect on clearance for the control, bevacizumib and sunitinib groups, effect that were not observed with everolimus. This is not fully understood and would require further control experiments. The on-going histology may also provide further insights into the mechanisms involved.

REFERENCES 1. Kircher, et al., Radiology 2012, 263(3): p. 633. **2** Poirier-Quinot, et al., '*MRI: recent advances and new horizons'*. In Molecular Imaging Techniques: New Frontiers. Future science 2013.