Evaluation of the reversibility of the binding between a targeted CA and its receptor by in vitro micro-MRI

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INTRODUCTION

Nowadays, high-molecular-weight contrast agents (CA), such as nanoemulsions, which can carry several thousands of Gd^{3+} ions are developed to overcome the low sensitivity of MRI. To assess the specificity of such targeted CA, conventional *in vitro* tests consist in incubating the CA with cells that overexpress the targeted receptor and then imaging the cell pellets with appropriate sequences. However, these methods do not allow to assess the reversibility of the binding between the CA and the receptor. Here, we propose a new technique based on our previous work [1] that enables to control the contrast mechanism as a function of the biodistribution of the targeted CA**.**

MATERIAL AND METHODS

Cells: A monolayer of HUVEC cells which overexpress the $\alpha_v\beta_3$ integrins was obtained by seeding 280 000 cells in the microfluidic channel (dimensions 0.4×5×50mm^3) of a µ-Slide I 0.4 Luer (Ibidi, Germany).

MRI: Experiments were carried out on a 2.35 T scanner (Bruker, Germany). A T_1 weighted 3D FLASH sequence was applied with 12.4µm resolution perpendicular to the cell layer (cell layer's thickness is about 10 µm), an in-plane resolution of 200×400 µm^2, TR/TE = 75/3.7ms. Unidimensional signal profiles were extracted by projection of the image matrix along planes parallel to the cell layer as previously described in [1].

Protocol: The protocol was inspired by the BIAcore system [2]: a flow of an $\alpha_v\beta_3$-specific paramagnetic emulsion (nanoparticle concentration : 3.75 nM) was applied during 30 minutes over the cell layer using a syringe pump and silicon tubing, with a velocity comparable to the blood's one in capillaries [3]. At 15 and 30 minutes, the flow was stopped and the medium containing the nanoemulsion over the cell layer was flushed and replaced with CA free medium; images were then acquired. After these 30 minutes, a flow of CA free culture medium was applied over the cells and images were acquired every 10 minutes for 70 minutes.

RESULTS & DISCUSSION

As previously described in [1], when the flow of nanoemulsion is applied over the cell layer, a part of the CA binds specifically to the $\alpha_v\beta_3$ integrins inducing a signal enhancement on the cell layer of about 25 % (figure 1). The figure 2 shows the relative signal enhancement as a function of time. After 30 minutes, only culture medium is applied over the cell layer and the signal enhancement decreases until 11% after 70 minutes. That decrease may be due to the dissociation of the binding between the targeted CA and the $\alpha_v\beta_3$ integrins, an internalization of the CA within the cells leading to a decrease of its apparent relaxivity and probably other mechanisms which have to be identified. Based on the relative enhancement curves and the theoretical formula of the SPGR signal, we calculated the apparent T_1 of the cell layer and hence the concentration of CA linked to the receptors. The kinetics of the CA concentration was adjusted with a monoexponential function, which led to a value of a time constant of 0.0121 min^{-1}.

CONCLUSION

In this study, we introduced a new method to characterize the binding kinetics of a contrast agent *in vitro*. Optical imaging techniques may be useful to confirm the biodistribution of the CA. Inspired by the BIAcore system, this technique mimicked *in vivo* conditions in terms of CA concentration and velocity of the flow. Knowing the kinetics of a CA towards its receptor could help optimizing the *in vivo* protocols especially for the optimal time between the injection of the CA and the imaging acquisition.

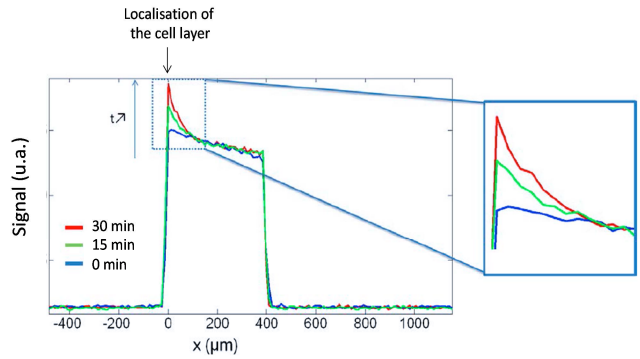

Figure 1: Unidimensional profiles at times 0, 15, 30 min after the application of a flow of paramagnetic emulsion over the cell layer

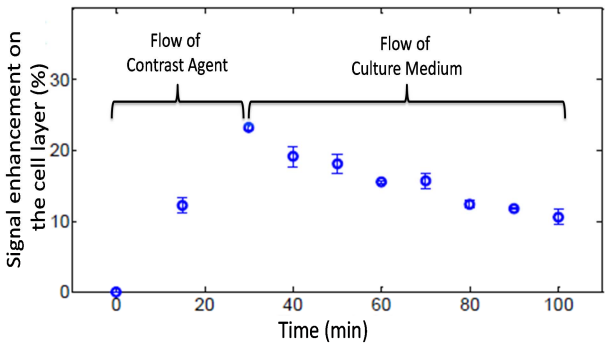

Figure 2: Relative signal enhancement on the cell layer as a function of time. During the 30 minutes, a flow of emulsion is applied. The emulsion is then replaced with CA free culture medium for the next 70 minutes.

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3. Stücker, M et al, Microvascular Research, 1996. 52 : p.188-192