Targeting of integrins with a new nonpeptidic RGD mimetic grafted to USPIO

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Introduction. The cell-cell and cell-matrix interactions are mediated, in part, by the ubiquitously expressed class of cell surface receptors known as integrins. These receptors represent interesting therapeutic targets because of their important role in diverse pathologies, i.e. restenosis, atherosclerosis, acute renal failure, tumor-induced angiogenesis and metastasis formation. The tripeptide sequence RGD (Arg-Gly-Asp) is a common cell-recognition motif, which is part of integrin binding ligands, like fibronectin, fibrinogen, and vitronectin [1]. The integrin targeting with RGD containing molecules has extensively been explored for therapeutic [2] or diagnostic [3] purposes. In our work, a new contrast agent has been synthesized by grafting on USPIO a nonpeptide small molecular weight RGD mimetic (USPIO-g-mimRGD) [2]. Its efficacy to target integrins has been tested *in vitro* on Jurkat cells. The results were compared with homologous contrast agents, which contained the GRGD peptide or the CS1 fragment of Fibronectin grafted on USPIO (USPIO-g-GRGD, USPIO-g-FN).

Material and method.

The RGD mimetic (figure 1) was obtained as described by Sulyok [2] and was grafted on the surface of superparamagnetic nanoparticles in two steps : the dextran coating of USPIO was treated with epichlorhydrin then reacted with the mimetic.

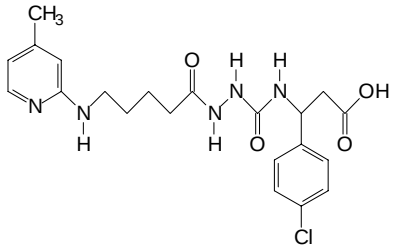

Figure 1: Structure of the RDG mimetic

The diagnostic potential of the new contrast agent was tested *in vitro* by using a method (C-MALISA, cellular magnetic-linked immunosorbent assay) developed in our laboratory [4]. Briefly, Jurkat cells were stimulated with phorbol myristate acetate (PMA). The cells were suspended in buffered formalin and fixed on ELISA plates. After incubation for 3 hours at 37°C, the contrast agents bound to the cells were digested with 5 N HCl and the samples were analyzed by MRI (Bruker AVANCE-200, 4.7 T, TR/TE = 3000/20-2000 ms). USPIO particles were used as control. The T_2 of water protons measured on images were correlated with [Fe] determined by Prussian blue.

Results. The significant difference between the stimulated cells incubated with USPIO-g-mimRGD, USPIO-g-GRGD or USPIO-g-FN and the control ones demonstrate the specific interaction of the two contrast agents with integrins (Figure 2).

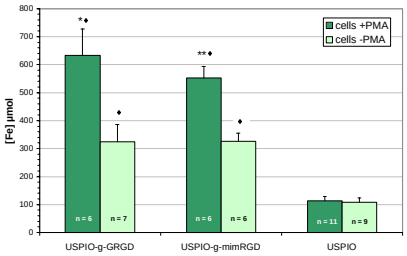

Figure 2. Comparison between USPIO-g-mimRGD and USPIO-g-GRGD bound on Jurkat cells stimulated or not with PMA

Conclusions. The results prove that USPIO-g-mimRGD is an efficient integrin-targeted contrast agent. The new compound is the first non-peptide RGD mimetic grafted to USPIO for diagnostic purposes, which can find a wide range of applications for the MRI detection of pathologies like atherosclerosis or cancer.

References

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