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Control/Tracking Number : 03-M-449-ESMRMB **Activity :**Scientific Paper (Methodology)
Current Date/Time : 5/6/2003 1:24:24 AM **C-MLISA (Cellular Magnetic-Linked Immunosorbent Assay), a New Application of Cellular ELISA for MRI**

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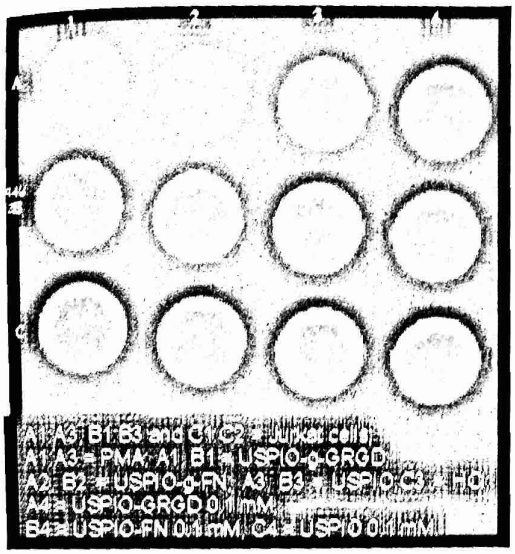
Introduction: The high throughput MRI has already been used [1] for the evaluation of the targeted nanoparticles. A modified cellular ELISA (C-MLISA) has been developed in the present work as an MRI application for clinical diagnosis. Two contrast agents with affinity for VLA-4 (very late antigen-4) integrin were synthesized by grafting the peptide GRGD and the CS1 fragment of Fibronectin (USPIO-g-GRGD, USPIO-g-FN) to USPIO particles. Both contrast agents were tested by C-MLISA on Jurkat cells and on rat mononuclear cells (MNC), which were stimulated to activate their integrins. Such an application presents interest for the in vitro detection of inflammatory pathologies, i.e. cancer, atherosclerosis.

Subjects and Methods: Jurkat cells were stimulated with phorbol myristate acetate (PMA). Hepatitis was induced to Wistar rats with Concanavalin A, and the blood collected to isolate the MNC by Histopaque density gradient. The cells were suspended in buffered formalin and fixed on ELISA plates, which were subsequently blocked with a blocking buffer. The cells were incubated with the contrast agents (USPIO used as control) for 3 hours, and rinsed again with HBSS. The contrast agents bound to the cells were either digested with 5 N HCl, or resuspended by competition with the peptide. The specificity of the contrast agent for integrins was tested by pre-incubating the cells with the peptide. A calibration curve was constructed by incubating the cells with different concentrations of the contrast agent. The samples were analyzed by MRI (Bruker AVANCE-200, 4.7 T, TR/TE = 3000/20 ms, 20 echoes), and the T_2 measured on images. Fe concentration was determined in each sample with Prussian blue, and the values correlated with T_2.

Results: The images (figure) show a striking difference between the stimulated cells incubated with USPIO-g-GRGD or USPIO-g-FN and the control ones. The significant correlation between T_2 and [Fe] (r^2 for cells +PMA = 0.987; r^2 for cells -PMA = 0.998) demonstrate the specific interaction of the two contrast agents with integrins (i.e. VLA-4) (table).

This specificity is confirmed by the pre-incubation of the cells with the peptide, which inhibits the binding of the contrast agent at the receptor sites.



Treatment of cells	USPIO-g-GRGD		USPIO-g-FN		USPIO	
	T_2 (ms)	[Fe] µM	T_2 (ms)	[Fe] µM	T_2 (ms)	[Fe] µM
+PMA	85.1	1060	79.2	901.5	163.4	373.9
-PMA	184.6	327.9	171.0	294.9	333.5	76.7

Conclusion: C-MLISA shows promise as a new MRI method for the in vitro diagnosis of various pathologies (i.e. inflammatory), and could find application for the validation of specific contrast agents.

References:

1. Högemann D, Ntziachristos V, Josephson L [2002] Bioconjugate Chem 13: 116-121.

Topic (Complete): 211 Cells, Extracts, Fluids **Additional (Complete):**

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