A novel Gd-based MRI Contrast Agent responsive to the Factor XIII transglutaminase activity

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Introduction

Fibrin stabilizing Factor XIII is involved in many pathologies, such as thrombotic disorders, coronary artery disease, myocardial infarction and cerebrovascular disease.[1] The MRI "in vivo" visualization and quantitation of the Factor XIII activity may be a very useful tool for the diagnosis of these pathologies and the monitoring of therapeutic follow-up . Plasma Factor XIII is a heterologous tetramer composed of two A and two B subunits. The 50% of the total fibrin stabilizing activity in blood is found in platelets where Factor XIII exists as A-subunit dimer. Thrombin cleavage of A-subunit is necessary to activate the plasma tetramer and dimeric platelet Factor XIII. Calcium ions are also required for the activation of plasma Factor XIII after thrombin cleavage, and for the first catalytic step. Activated Factor XIII (Factor XIIIa) is a transglutaminase that does not contribute to the process of clotting of blood, but instead stabilizes the clot cross-linking proteins in the fibrin thrombus by forming an isopeptide bond between the γ-carbonyl group of a glutamine in one protein and the ε-amino group of a lysine residue in a nearby protein.[2] Moreover, elevated activity of transglutaminases was shown at the boundaries of invading tumors and several biological events such as cellular proliferation, differentiation and apoptosis seem regulated by such enzymes.[3]

Methods

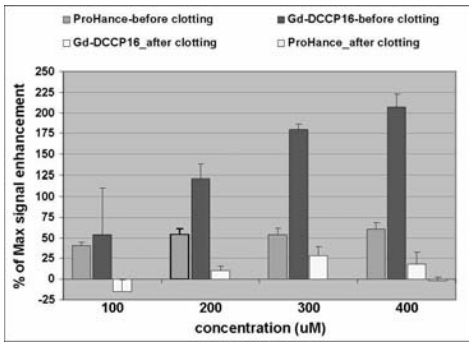
Using molecular mechanics and docking computational methodologies we investigated the quaternary structure of Factor XIII and its catalytic centre with the aim of designing a Gd-containing peptide able to bind the protein and crosslink with fibrinogen. Predictive calculation designed the peptide DCCP16 able to interact with the catalytic centre of the enzyme.

The Gd-containing peptide (Gd-DCCP16) was synthesized by solid phase peptide synthesis, purified by HPLC and characterized by MS and NMR spectroscopy. The Gd(III) complex of DCCP16 was formed in water in presence of $GdCl_3$.

A peptide based probe for MR-imaging of Factor XIII activity, extracted from α_2-antiplasmin, the primary inhibitor of plasmin-mediated fibrinolysis, was already published in the literature.[4] We have therefore tested "*in vitro*" our Gd-DCCP16 against GdHPDO3A as negative control and also against the already published imaging probe. Clots were formed by mixing GdDCCP16, a coagulation coadjutant and $CaCl_2$ solution after incubation at 37 °C for 30 min.

Results

A marked signal enhancement has been observed when Gd-DCCP16 is present during the clot formation whereas a much reduced effect is detected when the contrast agent is added to an already formed clot. The signal enhancement in the NMR image is therefore only due to the formation of covalent bonds between peptide and fibrin. Only a small signal enhancement was observed when GdHPDO3A was used instead of GdDCCP16. These tests also demonstrated that our probe give a higher signal enhancement (about 70% increase at 0.4 mM concentration of the probes) in respect to the already published imaging probe.



Conclusions

Gd-DCCP16 appears a good candidate as MRI contrast agent responsive to the activity of Factor XIII. It is expected that the "*in vivo*" evaluation of the activity of this enzyme may help to visualize microthrombi formation in tumours, in vascular and cerebrovascular diseases, as well as proangiogenic processes.

References

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