Diagnostic activity of a new targeted theranostic agent for the peri-infarct region in stroke

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Introduction

A key target for stroke treatment is the peri-infarct region, a no man's land between severely affected tissue (infarct core), with a spreading front of mediators of damage, and unaffected (healthy) tissue, with mediators of remodeling and recovery. Nanotechnology provides a unique framework to develop theranostic molecules that target specific tissues, to act as imaging probes and therapeutic entities. In this abstract we report the identification of specific molecular markers of the peri-infarct tissue in stroke, and the development of a new theranostic agent, which specifically targets cells of the peri-infarct area of the ischemic brain. We demonstrate its capacity to act as (multimodal) imaging probe, for the identification of cells at the peri-infarct region.

Material and Methods

Liposome-based theranostic agents were constructed with DSPC, Cholesterol, DSPE-PEG, Rhodamine-PE and Gd-DTPA-BSA, by using the lipid film hydration and extrusion method. Targeting antibodies were coupled to liposomes by maleimide-DSPE-PEG groups. As animal models of stroke we used the intraluminal transient (90 min) occlusion, or a permanent transcranial suture, of the MCA in male SD rats. Induction of heat shock proteins' expression on astrocytes and HUVEC cells (in vitro models of peri-infarct region) was achieved by heating (42 ºC, 30 min) following by a resting period of 6h. MRI studies were conducted on a 9.4T MRI system (Bruker Biospec). Parametric maps were constructed from T1 (saturation-recovery) and T2 (mono-exponential decay) images using Image-J. Proteomic studies of brain tissues from ischemic rats were performed by 2D-PAGE and COFRADIC techniques. Protein identification was performed by mass spectrometry. Wester-blot and immuno-histochemical analysis were also performed.

Results and Discussion

The key factor for the development of a theranostic agent is the definition of a proper molecular marker at the target tissue. In figure 1 we see part of our multidisciplinary study (proteomics, wester-blot and immuno-histochemistry) in ischemic brains of rats. Ischemic core (stained by TTC), peri-infarct region, and healthy (control) tissues were analyzed. This study provided 15 suitable candidates as molecular markers of peri-infarct tissue, all highly over-expressed on that region (HSP70 family was the most expressed one). Only one among all them, a minor sub fraction of the family of HSP70 proteins (as determined by 2D-western-blot studies) was specific for the peri-infarct tissue, and selected as target.

In molecular recognition studies by incubation (for 30 or 60 min) of cells over-expressing the family of HSP70 proteins (model for peri-infarct tissue) and targeted and non targeted multimodal imaging probes (Fig 2), we observed that only cells expressing the target, and incubated with targeted contrast agents showed a significant fluorescence (Fig 2, top-left) and reduced T1 relaxation times on MRI (Fig 2, [1]), as compared to stressed cells incubated with non- targeted theranostic agent (Fig 2, bottom-left and [2]), or non-stressed cells incubated with targeted (3) or non-targeted (4) imaging probes. Culture medium was used as control for MRI.

Conclusions

We have proved the diagnostic activity of the designed theranostic agent, which specifically interacts with cells of the peri-infarct region in stroke, allowing its identification by MRI and fluorescence microscopy.

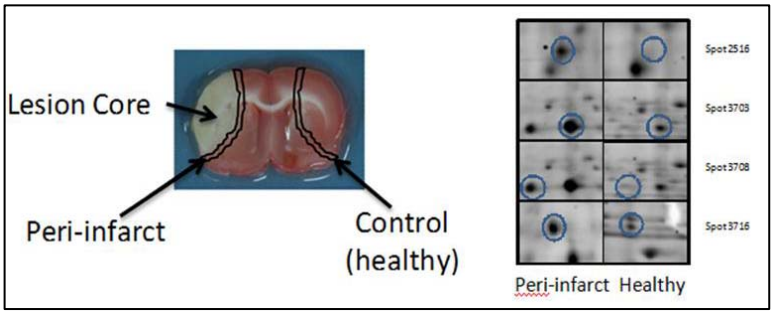

Figure 1. Proteomic studies of peri-infarct tissue targets

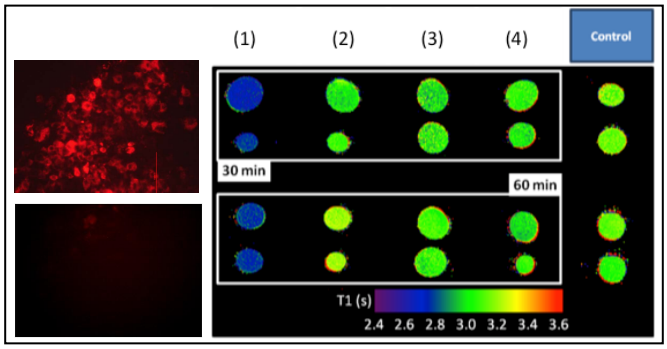

Figure 2. Fluorescence and MRI of cells-probe interaction

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