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

Jesús Agulla[1], David Brea[1], María Pérez-Mato[1], Miguel Blanco[1], José Castillo[1], and Pedro Ramos-Cabrer[1]

[1]*Clinical Neuriosciences Research Laboratory, Department of Neurology, University Clinical Hospital, University of Santiago de Compostela, Santiago de Compostela, Spain*

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 use of a new theranostic agent, which specifically targets cells of the peri-infarct area of the ischemic brain, for the treatment of stroke.

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. The molecular target (a minor fraction of the HSP70 protein family) was previously determined by proteomic studies As animal models of stroke we used the permanent transcranial suture of the MCA in male SD rats (300-350g). Groups of study (n=6) were: (1) saline treated, (2) free citicoline i.p., (3) free citicoline i.v., citicoline encapsulated in (4) non-targeted and (5) targeted liposomes. MRI studies were conducted on a 9.4T MRI system (Bruker Biospec). DWI (<30 min) and T2 weighted images (day 1, 3 and 7 after stroke) were acquired and processed using Image-J, determining lesion volumes and edema formation for all animals. Immuno-histological studies of the expression of the target protein at the peri-infarct region was also performed

Results and Discussion

We have previously developed a theranostic agent that specifically targets the peri-infarct region in stroke. This agent was doped with citicoline, a known neuroprotectant. In order to assess the best timing for the administration of the theranostic drug, we conducted an immune-histological analysis of the expression of the target on the ischemic brain of rats (from 30 min up to 14 days after stroke) and found that the target protein was significantly expressed in the brain up to 7 days, with maximal expression at days 1-2 (figure 1). Thus we performed an in vivo study of the therapeutic effect of citicoline administered free (i.p. and i.v.) and encapsulated in non-targeted and targeted liposomes, using repetitive injections (every 6 h) during the first 30 hours, following induction of ischemia. Total administered citicoline was 500 mg/kg (i.p. route) or 48 mg/kg (i.v. route and liposome-encapsulated). We followed lesion volumes and edema formation by MRI at days 1, 3 and 7 (Figure 2). Initial volumes on DWI were not significantly different among all studied groups. Control animals (treated with saline) presented an increment on lesion volume of ca. 50%, at day 7, while animals treated with citicoline i.p. presented an increase of ca. 10%. On the contrary, animals treated with citicoline i.v., and targeted and non-targeted encapsulated citicoline (i.v.) experienced important reductions on lesion volumes at day 7 (ΔV: free citicoline i.v. < non-targeted < targeted liposomes). Effects were observed at earlier stages (day 1 and 3) for encapsulated citicoline. No effects on edema formation were observed.

Conclusions

We have proved the therapeutic capacity of the designed theranostic agent, which specifically interacts with cells of the peri-infarct region in stroke, improving the effectiveness of the neuroprotetive drug encapsulated in it.

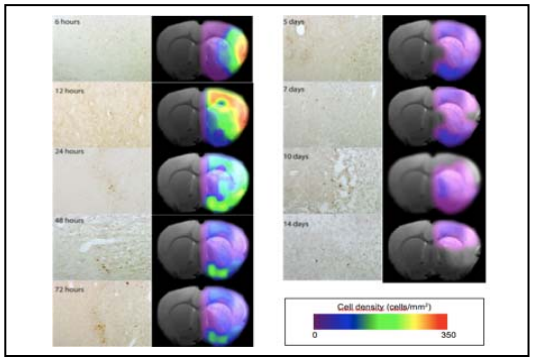

Fig 1. Temporal evolution of peri-infarct region

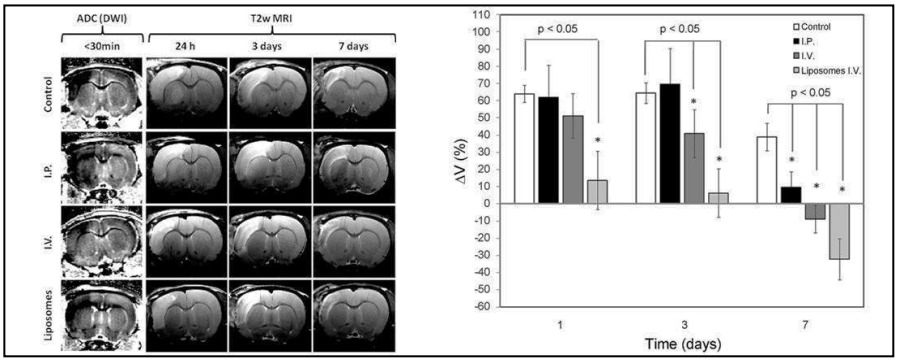

Fig 2. Lesion volumes after administration of free and encapsulated citicoline.

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