Post-ischaemic gene therapy for stroke: an MRI study.

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INTRODUCTION: The heat shock protein (HSP) family of molecular chaperones has an essential role in ensuring functional protein synthesis and accelerating cell repair after environmental stress (1). *In vitro* and *in vivo* studies have supported the neuroprotective role of stress-triggered HSPs, HSP27 and HSP70 in particular (2). This study uses MRI to assess the effects of post-ischaemic viral delivery of HSPs on lesion size in a rat middle cerebral artery (MCA) occlusion model of reversible focal ischaemia. Multislice T_2-weighted scans were used to determine lesion volume and perfusion maps were acquired to measure cerebral blood flow (CBF) at 6 different time points up to 4 weeks after stroke induction. Behavioural tests (bilateral asymmetry and foot-fault tests) were performed at the same time points as MRI in order to assess functional recovery.

SUBJECTS AND METHODS: Sprague Dawley male rats (250-300g) were anaesthetised with 2% isoflurane in 100% O_2. Middle cerebral artery occlusion (MCAO) was performed by intraluminal insertion of a 290µm araldite-coated monocryl suture advanced 17mm from the carotid bifurcation. After 30 minutes the suture was completely withdrawn to reperfuse the tissue. Half an hour after reperfusion, viral suspensions (2.5µl of $2x10^6$ pfu) of herpes simplex virus carrying HSP27 (n=6), HSP70 (n=6) and LacZ (n=6) as a control were stereotactically microinjected into rat striatum. Rats were allowed to recover for 1 day and then scanned at 6 different time points after ischaemia: 24 hours, 72 hours, 1 week, 2 weeks, 3 weeks, and 4 weeks. Coronal images were obtained on a 2.35 Tesla horizontal bore SMIS magnet at approximately 0.5mm from bregma with a 40x20mm FOV, a 2mm slice thickness and 128x64 pixels. Multislice T_2-weighted SE images (1mm slice thickness, 9 slices, TE=120ms, TR=1500ms) were acquired for lesion definition, and T_1 and CASL (continuous arterial spin labelling) EPI sequences were acquired on a 2mm brain slice within the MCA territory for quantitative CBF mapping (3). Regions of interest were drawn to determine total lesion size per slice. All animals were imaged under general anaesthetic (2% halothane in a 70:30 N_2O:O_2 mix) with physiological monitoring (ECG, temperature). The bilateral asymmetry test gives an indication of sensory neglect and tactile extinction by measuring the time to removal of a sticky tape from both forepaws as an indication of sensory hemineglect. The foot-fault test was used to assess the animal's motor-coordination deficit over time by counting the total number of steps taken in 1 minute together with the number of left paw misplacements on the grid.

RESULTS: Figure 1 shows lesion volume per slice calculated from multislice T_2-weighted images obtained 2 and 4 weeks after MCAO and viral microinjection. Differences in lesion size between the groups were apparent in both basal ganglia and cortex up to 4 weeks after ischaemia. End point measurements of total lesion volume (mean ± se) for all three groups were 48.5 ± 8.3mm^3 for HSP27 injected rats, 68.9 ± 8.3mm^3 for HSP70 injected rats and 71.5 ± 8.3mm^3 for LacZ injected animals. A significant difference in lesion volume of 23.0 ± 11.8mm^3 ($p = 0.05$) was found between HSP27 treated animals and controls, whereas HSP70 treated animals showed no reduction in lesion size compared to controls. A regional analysis was performed on the multislice data by grouping frontal slices (1, 2), mid MCA territory slices (3, 4, 5, 6) and posterior slices (7, 8, 9). This analysis suggested that HSP27 treatment was more effective in the posterior region than in the frontal and mid regions. CBF data indicated that the tissue was reperfused above ischaemic levels in all groups at all timepoints. HSP27 treated animals removed the sticky tape from their left, affected paw faster than controls over time. Moreover, these animals showed a higher percentage of correct left forepaw placements on the grid in the foot-fault test at all timepoints compared to LacZ injected controls. No differences in behavioural performance were observed between HSP70 treated animals and controls (figure 2).

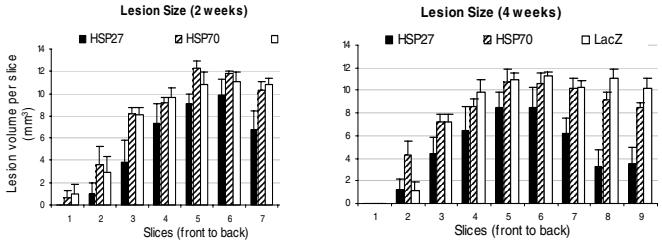

Figure 1. Mean lesion volume per slice at 2 and 4 weeks after stroke and viral microinjection with HSV-HSP27, HSV-HSP70 and HSV-LacZ.

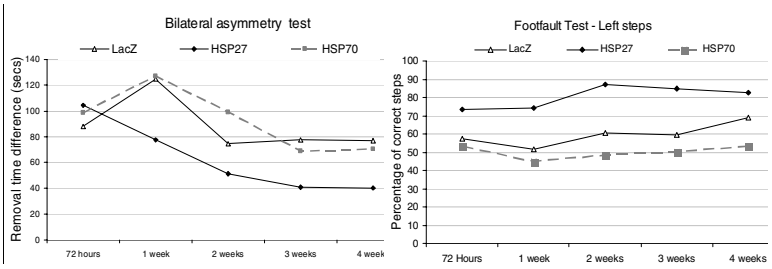

Figure 2. Mean performance on behavioural tests at all timepoints after stroke in HSP27 treated, HSP70 treated and LacZ injected controls.

DISCUSSION AND CONCLUSION: Pre-ischaemic treatment with HSP27, and not HSP70, has been shown to have a significant effect on lesion size after MCAO (4). Consistent with this, our results suggest that post-ischaemic gene delivery of HSP27 causes a reduction in lesion size that can be detected by MRI up to one month after induction of reversible ischaemia. These animals also showed functional recovery in the bilateral asymmetry test and reduced behavioural deficit on the foot-fault test over time compared to controls. HSP70 overexpression appeared to have no positive effect on lesion size or functional recovery as compared to controls. In summary, non-invasive MRI techniques were combined with behavioural tests to demonstrate the long term post-ischaemic neuroprotective effects of HSP27 and not HSP70 in a rat model of reversible focal cerebral ischaemia.

REFERENCES: (1) Richter-Landsberg C, Goldbaum O (2003) *Cell Mol. Life Sci.* **60**, 337-349; (2) Kelly S, Yenari M (2002) *Curr. Med. Res. Op.* **18**, 55-60; (3) Alsop DC, Detre JA (1996) *J Cereb. Blood Flow Metab.* **16**, 1236-1249; (4) Aron Badin R *et al.* (2005) *J Cereb. Blood Flow Metab.* (in press).