GENE THERAPY FOR STROKE: AN MRI STUDY.

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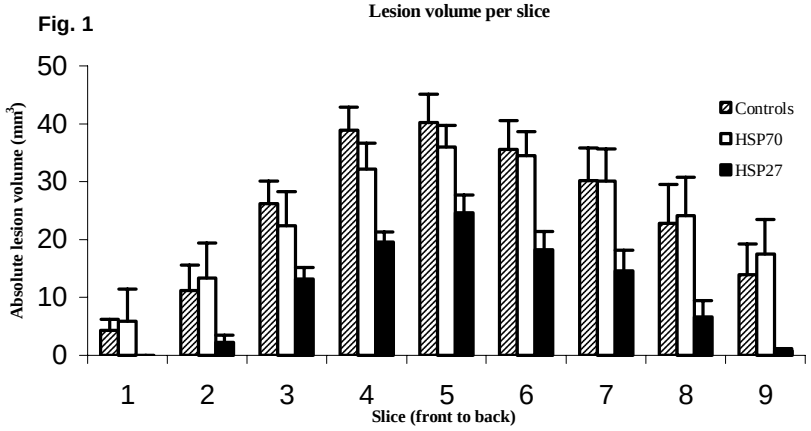
INTRODUCTION

Heat shock proteins (HSPs) are molecular chaperones with essential roles in cellular function such as modulation of the proteolytic machinery and acceleration of cell repair (1). *In vitro* and *in vivo* studies have supported the neuroprotective role of stress-triggered HSPs (2). This study uses MRI to assess the effects of pre-ischaemic viral delivery of HSPs on lesion size in a rat middle cerebral artery (MCA) occlusion model of reversible focal ischaemia. Perfusion maps of a 2-mm brain slice within the MCA territory were acquired to measure cerebral blood flow (CBF) and multislice T_2-weighted scans were used to determine lesion volume 24 hours after ischaemia.

SUBJECTS AND METHODS

Sprague Dawley male rats (250-300g) were anaesthetised with 2% isoflurane in 100% O_2. Viral suspensions (2.5 µl of $2x10^6$ pfu) of herpes simplex virus carrying HSP27 (n=6), HSP70 (n=6), or LacZ (n=6) as a control, were stereotactically injected into rat striatum (1). Three days post-injection, rats were re-anaesthetised for middle cerebral artery occlusion (MCAO) by intraluminal insertion of a 290-µm-suture advanced approximately 17 mm from the carotid bifurcation. After 30 minutes the suture was completely withdrawn to reperfuse the tissue and rats were allowed to recover for 24 hours before scanning. Coronal images were obtained approximately 0.5 mm from bregma on a 2.35 Tesla horizontal bore SMIS magnet with 40x20 mm FOV, 2 mm slice thickness and 128x64 pixels. T_1 and CASL (continuous arterial spin labelling) EPI sequences were run for quantitative CBF mapping (3) and multislice T_2-weighted SE images (1mm slice thickness, 9 slices, TE=120 ms, TR=1500 ms) were acquired for lesion definition. All animals were imaged under general anaesthetic (halothane 2% in a 70:30 N_2O:O_2 mix) with physiological monitoring (ECG, rectal temperature). Three days later, the brains were extracted and immunohistochemistry and Western blots were carried out in order to verify expression levels of virally delivered HSPs in the brain.

RESULTS



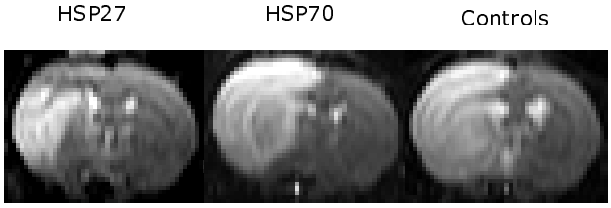


Figure 2. Representative centre slice T_2-weighted images of treated and control animals obtained 24 hrs after occlusion of the MCA. Intracerebral microinjections into the rat striatum with HSV-HSP27, HSV-HSP70 and HSV-LacZ were performed three days before ischaemia. Lesion area appears as bright signal on T_2-weighted images. Pre-ischaemic HSP27 treatment reduced lesion size in the cortex and basal ganglia.

Figure 1 shows lesion volume per slice calculated from multislice T_2-weighted scans obtained 24 hours after reperfusion. Representative centre slice images (0.5 mm from bregma) of the multislice data sets illustrate differences in lesion size in HSP27 treated, HSP70 treated and LacZ controls (Figure 2). Total lesion volume was reduced by 44.8% (p=0.02) in HSP27 treated animals compared to controls whereas no significant differences were found between HSP70 treated and control animals (p=0.88). CASL maps indicated that there was no significant difference in relative CBF 24 hours after reperfusion in normal and ischaemic hemispheres between the groups that could account for differences in lesion size. Histological analysis of brain sections showed widespread staining for HSPs in basal ganglia and cortex. Western blots performed 72 hours after MCAO revealed that levels of HSP expression in HSP injected hemispheres were 4 times higher than in LacZ injected controls.

CONCLUSIONS

HSP27 treatment resulted in a significant reduction in lesion size after MCAO as calculated from multislice T_2-weighted images. Virally induced overexpression of HSPs was histologically detected in both treated groups but only showed a neuroprotective effect in HSP27 injected animals. In conclusion, we show that non–invasive MRI techniques can detect a significant reduction in lesion size after HSP27 gene delivery in a rat model of reversible focal cerebral ischaemia.

(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.