Effect of heat shock protein overexpression in a mouse stroke model

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Introduction Heat shock proteins (HSPs) have been reported to increase cell survival in response to a wide range of cellular challenges. In order to investigate the mode of action of these proteins *in vivo*, transgenic (tg) mice overexpressing HSP27 or HSP70 have been compared to wild-type (WT) mice in a middle cerebral artery occlusion (MCAO) model of permanent cerebral ischaemia. To assess the contribution of HPSs following permanent ischaemia, the total lesion size was estimated after 24 hours of ischaemia using a multi-slice T2-weighted scan.

Methods 4 groups of mice were used: 1. HSP27 tg (n=5), 2. HSP27 WT (n=6), 3. HSP70 tg (n=5) and 4. HSP70 WT (n=6). All mice and 5 WT mice were anaesthetised with 2.5% isoflurane and maintained on 1.75% isoflurane with pure oxygen. The MCA was permanently occluded by advancing a 180-µm-diameter filament into the internal carotid artery past the MCA junction. Coronal images were obtained 24 hours after the onset of stroke. A 2.35T horizontal bore SMIS MR scanner was used with the following imaging parameters: T2-weighted SE sequence with FOV 20 mm, 1 mm slice thickness, 9 slices and 128 × 64 pixels. The relative infarcted area per slice was defined as the ratio of the lesion area to the whole brain size. All data are presented as mean ± SD.

Results

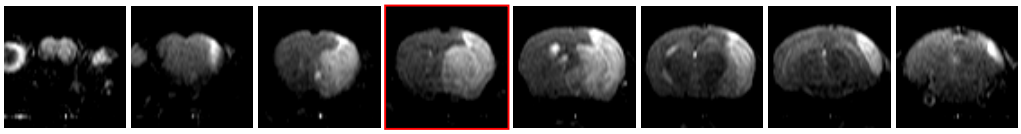

Fig. 1. Example of T2-weighted images at 24 hours after stroke for a single mouse, showing the lesion area as bright signal. The MCA slice has a red border.

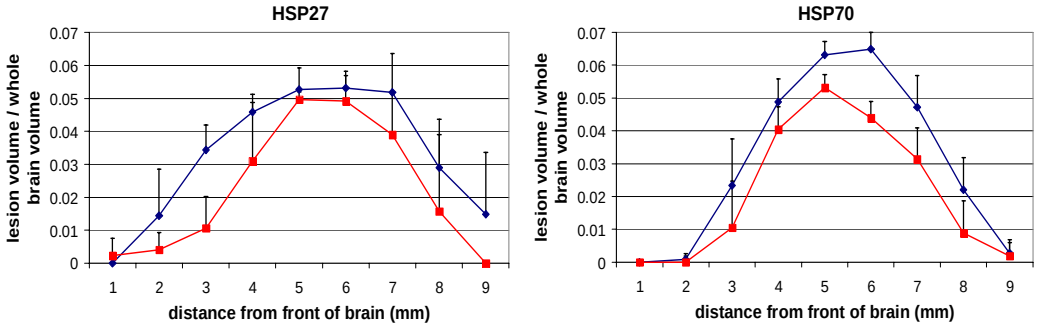

Fig. 2. A. Relative infarcted area for HSP27 tg and HSP27 WT mice. B. Relative infarcted area for HSP70 tg and HSP70 WT mice. Blue: WT mice. Red: tg mice.

Figure 1A shows a typical infarct caused by permanent MCA occlusion. The relative infarcted area (Fig. 2) is larger in the WT mice than in the HSP27-overexpressing animals for every slice ($P < 0.05$ using degrees of freedom adjusted repeated measures ANOVA). The same is true for HSP70-overexpressing mice, as reported earlier.[1] Overall, the lesion is 30 ± 3 % and 27 ± 4 % of the whole brain volume in the HSP27 WT and HSP70 WT mice respectively, compared to 20 ± 7 % and 19 ± 6 % in the HSP27 tg and HSP70 tg animals. Western blots showed that HSP27 and HSP70 were highly expressed in HSP27 tg and HSP70 tg mice respectively.

Discussion We have imaged lesion size in a permanent MCAO model to investigate whether heat shock proteins can protect the brain against ischaemia. With this particular model, the lesion is significantly smaller for both HSP27 and HSP70 overexpressing mice compared to WT animals. Interestingly, similar work using HSP27 or HSP70 gene delivery via a viral vector in a rat model of transient MCAO showed protection for HSP27, but not for HSP70 (personal communication).[2] The difference between these experiments is probably partly due to the amounts of HSP overexpression and their spatial distribution, which are not the same in the transgenic mouse model and the viral delivery rat model. Furthermore, the different MCAO models (permanent versus transient) are known to influence the amount of apoptosis expected in infracted tissue, and thus the differences between the two models may reflect the different inhibitory actions of HSP27 and HSP70 in the apoptotic cascade.

References Van der Weerd et al. Neuroprotective effects of HSP70 after stroke – an MRI study. ISMRM 2004, 1450.
Aron-Badin et al. Neuroprotective effect of virally delivered HSPs in experimental stroke, submitted.

Acknowledgments We thank the BBSRC and Wellcome Trust for financial support of this project.