Response to letter by Yao

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Response:

We thank Dr Yao for his interest and comments on our article[1]. However, we challenge the points raised as possible pitfalls. 1. MABP. For MRI scanning, SHRSP were anaesthetised (2-3% isoflurane to immobilise) and ventilated. MABP was 93±4mmHg. It is inappropriate to compare this with MABP recorded under 0.5% halothane and neuromuscular blockade [2], as Yao suggests. With this regime MABP will be higher but the animal may be conscious, the stress induced contributing to hypertension. Our SHRSP and WKY rats come from an established colony (20 years) within Glasgow University. Colony integrity and hypertensive phenotype are maintained by selecting SHRSP adult breeders with conscious systolic blood pressure (SBP) between 170-190mmHg (males) and 130-150mmHg (females) measured by tail cuff (TC). 12-16 week old SHRSP progeny have conscious SBP of 187mmHg compared to 134mmHg for age-matched WKY (comparable with values for Charles River (Wilmington, MA) [3] and Medical College of Georgia, originally University of Michigan, Ann Arbor) [4]). Japanese colonies generally demonstrate much higher baseline BP's. Divergence of BP phenotypes between distinct SHRSP colonies is not unexpected considering different husbandry regimes (including Na^+ content of diet) and criteria for selecting breeding pairs.

2. Age of rats. We believe the age range used in our study is appropriate. Using TC and

telemetry, SHRSP start to develop hypertension from 6-8 weeks and by 12-16 weeks SBP levels out and significant differences in conscious BP are evident between SHRSP and WKY [5,6].

Yao's point that infarct volume is higher in 5 month versus 10-12 week old SHRSP is interesting but may not be pertinent for proximal MCAO. Further, in the current study the comparison was between SHRSP and age-matched WKY.

3. We disagree with Yao's statement that stroke-resistant SHR are a better model for this study (more stable in terms of lower mortality). Experimental stroke is known to be more severe in SHRSP compared to WKY, their normotensive control [7], but we did not experience any significant mortality issues. Increased stroke sensitivity is likely to be due not just to genetically-determined hypertension, but include additional genetic factors influencing cerebral vessels, oxidative stress levels and insulin resistance.

4. Penumbra. We concluded that SHRSP (not SHR as Yao states) had limited penumbra (based on PWI/DWI mismatch area) one hour after stroke, significantly smaller than WKY. We acknowledge the limitations of PWI/DWI mismatch for detecting penumbra since in both animal and human stroke some of the diffusion abnormality can be reversed upon early reperfusion. This could partly explain greater tissue rescue in the SHR study [8]. The stroke model used [8] may generate a greater % of penumbra: distal MCAO induces ischaemia in cortex, which has a collateral supply, while ischaemia induced by proximal MCAO [1] includes striatal end artery tissue resulting in a greater % of ischaemic core. The important point is that mismatch area in core MCA territory was significantly larger in WKY compared to SHRSP, which led us conclude that SHRSP have less potentially salvageable tissue.

References

[1] McCabe C, Gallagher L, Gsell W, Graham D, Dominiczak AF, Macrae IM. Differences in the evolution of the ischemic penumbra in stroke-prone spontaneously hypertensive and Wistar-Kyoto rats. Stroke. 2009 Dec;40(12):3864-8. Epub 2009 Sep 24.

[2] Yao H, Cui ZH, Masuda J, Nabika T. Congenic removal of a QTL for blood pressure attenuates infarct size produced by middle cerebral artery occlusion in hypertensive rats. Physiol Genomics. 2007 Jun 19;30(1):69-73.

[3] Brosnan MJ, Carkner RD. Hepatic effects of a fructose diet in the stroke-prone spontaneously hypertensive rat. *Am J Hypertens.* 2008;21(6):708-714.

[4] Rigsby CS, Pollock DM, Dorrance AM. Spironolactone improves structure and increases tone in the cerebral vasculature of male spontaneously hypertensive stroke-prone rats. Microvascular Res. 2007;73:198-205.

[5] Koh-Tan HH, Graham D, Hamilton CA, Nicoll G, Fields L, McBride MW, Young B, Dominiczak AF. Renal and vascular glutathione S-transferase mu is not affected by pharmacological intervention to reduce systolic blood pressure. *J Hypertension*. 2009;27(8):1575-84.

[6] Graham D, McBride MW, Gaasenbeek M, Gilday K, Beattie E, Miller WH, McClure JD, Polke JM, Montezano A, Touyz RM, Dominiczak AF. Candidate genes that determine response to salt in the stroke-prone spontaneously hypertensive rat: congenic analysis. *Hypertension.* 2007;50(6):1134-41.

[7] Jeffs B, Clark J, Anderson N, Gratton J, Brosnan MJ, Gauguier D, Reid J, MacRae IM, Dominiczak AF. Sensitivity to cerebral ischaemic insult in a rat model of stroke is determined by a single genetic locus. *Nature Genet.* 1997; 16: 364–367.

[8] Yao H, Okada Y, Ibayashi S. Therapeutic time window for YAG laser-induced reperfusion of thrombotic stroke in hypertensive rats. *Neuroreport*. 2002;13:1005-8.