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References

- Alvarez JL, Dodelet-Devillers A, Kebir H, Ifergan I, Fabre PJ, Terouz S, et al. The Hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. *Science* 2011; 334: 1727–31.
- Dalmasso AP, Goldish D, Benson BA, Tsai AK, Wasiluk KR, Vercellotti GM. Interleukin-4 induces up-regulation of endothelial cell claudin-5 through activation of FoxO1: role in protection from complement-mediated injury. *J Biol Chem* 2014; 289: 838–47.
- Dohgu S, Takata F, Yamauchi A, Nakagawa S, Egawa T, Naito M, et al. Brain pericytes contribute to the induction and up-regulation of blood-brain barrier functions through transforming growth factor-beta production. *Brain Res* 2005; 1038: 208–15.
- Jackstadt R, Hermeking H. MicroRNAs as regulators and mediators of c-MYC function. *Biochim Biophys Acta* 2015; 1849: 544–53.
- Lee SW, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, et al. SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. *Nat Med* 2003; 9: 900–6.
- Musolino PL, Gong Y, Snyder JMT, Jimenez S, Lok J, Lo E, et al. Brain Endothelial dysfunction in cerebral adrenoleukodystrophy. *Brain* 2015.
- Pello OM, De Pizzol M, Mirolo M, Soucek L, Zammataro L, Amabile A, et al. Role of c-MYC in alternative activation of human macrophages and tumor-associated macrophage biology. *Blood* 2012; 119: 411–21.
- Podjaski C, Alvarez JL, Bourbonniere L, Larouche S, Terouz S, Bin JM, et al. Netrin 1 regulates blood-brain barrier function and neuroinflammation. *Brain* 2015; 138: 1598–612.
- Powers JM, Liu Y, Moser AB, Moser HW. The inflammatory myelinopathy of adreno-leukodystrophy: cells, effector molecules, and pathogenetic implications. *J Neuropathol Exp Neurol* 1992; 51: 630–43.
- Ronaldson PT, Demarco KM, Sanchez-Covarrubias L, Solinsky CM, Davis TP. Transforming growth factor-beta signaling alters substrate permeability and tight junction protein expression at the blood-brain barrier during inflammatory pain. *J Cereb Blood Flow Metab* 2009; 29: 1084–98.
- Smith AP, Verrecchia A, Fagà G, Doni M, Perna D, Martinato F, et al. A positive role for Myc in TGFbeta-induced Snail transcription and epithelial-to-mesenchymal transition. *Oncogene* 2009; 28: 422–30.
- Uto T, Contreras MA, Gilg AG, Singh I. Oxidative imbalance in nonstimulated X-adrenoleukodystrophy-derived lymphoblasts. *Dev Neurosci* 2008; 30: 410–8.
- Wang Y, Jin S, Sonobe Y, Cheng Y, Horiuchi H, Parajuli B, et al. Interleukin-1β induces blood-brain barrier disruption by downregulating Sonic hedgehog in astrocytes. *PLoS One* 2014; 9: e110024.
- Wosik K, Cayrol R, Dodelet-Devillers A, Berthelet F, Bernard M, Moumdjian R, et al. Angiotensin II controls occludin function and is required for blood brain barrier maintenance: relevance to multiple sclerosis. *J Neurosci* 2007; 27: 9032–42.

Let's be certain about sartans (and other potential new therapies for CNS injury)

This scientific commentary refers to 'Neurorestoration after traumatic brain injury through angiotensin II receptor blockage', by Villapol *et al.* (doi:10.1093/brain/awv172).

The vast majority of new drugs for the treatment of neurological injury that show efficacy in preclinical models fail in clinical trials. Accordingly, many groups instead choose to evaluate whether drugs that are already approved for other conditions can be repurposed for use in traumatic brain injury, stroke and spinal cord injury. One such group of drugs is the 'sartans', which are approved for the treatment of hypertension in many countries worldwide. Sartans are blockers of angiotensin II type 1 receptors (e.g. AT1R) but it

should be noted that they also bind to other receptors (e.g. PPARγ peroxisome proliferator-activated receptor gamma). Two examples of sartans are candesartan and telmisartan. Sartans have demonstrable neuroprotective and anti-inflammatory properties in animal models of stroke, although evidence from human trials is inconclusive at present (Bath and Krishnan, 2014).

In this issue of *Brain*, Villapol *et al.* present data indicating that candesartan and telmisartan can improve outcome after controlled cortical impact injury in mice when given acutely (up to 6 h afterwards). Genetic knockout of a receptor for sartans (AT1R) improves outcome after traumatic brain injury to a similar degree (Villapol *et al.*, 2015). These results

might suggest therefore that sartans also warrant evaluation following brain injury in humans.

The title of the paper by Villapol and co-workers refers to 'neurorestoration', which is a broad term that implies different mechanisms to different people. Benefits of sartans were observed up to 6 h after injury in wild-type mice, but not when given 24 h post-injury: a time frame compatible with a mechanism involving compensatory blood flow and neuroprotection. Consistent with this, both drugs did improve blood flow and reduce lesion volume when assessed 3 days after injury and treatment.

Interestingly, Villapol *et al.* used a dose of sartans that they state did not reduce blood pressure in wild-type mice. Blood pressure was

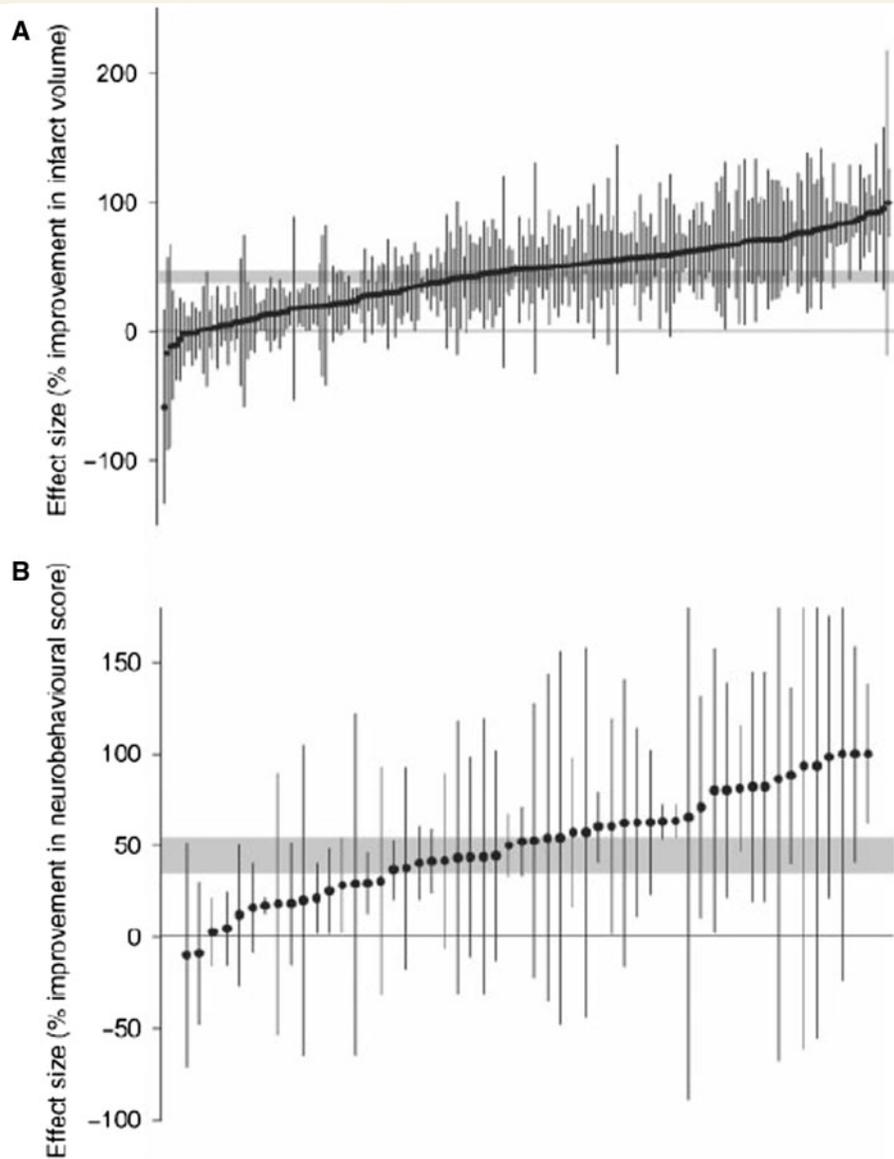


Figure 1 Point estimates and 95% confidence intervals ranked by effect size for (A) 222 comparisons testing change in infarct size and (B) 55 comparisons testing change in neurological score. Effect size is the improvement in hypothermic animals expressed as a proportion of the outcome in control animals. The grey band indicates the global estimate and its 95% confidence interval. The solid horizontal line marks where treatment and control are equal. Figure from van der Worp *et al.* (2007), used with permission and thanks to the authors.

measured 2 days prior to cortical impact ('baseline') and then 1 day after injury and treatment. However, at baseline (i.e. prior to treatment), the candesartan and telmisartan groups appear to have lower blood pressure than the vehicle control groups (see their Supplementary Table 1); as far as I can tell, this may have arisen by sheer bad luck during randomization. If these baseline differences are significant, then this confound means that one cannot

conclude that sartans improve outcome, although the study might still be held as evidence that lower blood pressure is associated with improved outcome. Consistent with this, the AT1R knockout mice had significantly lower blood pressure than wild-type controls and had smaller lesions and better neurological recovery. Considering the data from wild-type and knockout mice together, one might conclude that lower blood pressure is associated with

better outcome after traumatic brain injury.

However, if, as the authors report, sartans do provide benefits other than by lowering blood pressure, how do they work? Villapol *et al.* showed that lesion volumes were larger after combined delivery of a sartan plus an antagonist of PPAR γ than after either sartan alone (a 2% difference in lesion volumes). Thus, the authors argue that sartans improve outcome after traumatic brain injury via

Glossary

Hypertension: Abnormally high blood pressure.

Neuroprotection: Preservation of nervous system tissue or function by slowing disease progression or secondary cell death.

PPAR γ (and not by blood pressure reduction). However, there was some indication that this antagonist, when given by itself, increased lesion volumes by a similar degree (a difference of 2%; Fig. 5B), although in the 'Results' section the authors state that this was neither significant nor reproducible (their data not shown).

It is important to resolve the mechanism of action because many of the clinical trials using sartans for stroke have used doses deliberately designed to reduce blood pressure. There is evidence that high blood pressure after stroke leads to poorer outcomes, especially after haemorrhage, although it may not always be safe to reduce blood pressure after CNS injury (see references in Rothwell, 2015).

The authors state that 'Although to date sartans have not been adequately tested in neurodegenerative or traumatic brain disorders, there is clear clinical evidence that sartan administration is therapeutically effective in brain ischaemia and to prevent stroke'. This claim regarding stroke may need to be set in context. My (admittedly brief) examination of the clinical trial literature uncovered a Cochrane review meta-analysis from 2014 of 26 high-quality methodology clinical trials, which concluded that 'There is insufficient evidence that lowering blood pressure during the acute phase of stroke improves functional outcome' (Bath and Krishnan, 2014). However, many of the data arguing against a clinical benefit of sartans after stroke are from trials that intervened relatively late (up to 48 h after stroke) to reduce blood pressure. Moreover, there is some evidence for a clinical benefit of interventions that reduce blood pressure (by sartans and other methods) early after intracerebral haemorrhagic stroke: a *post hoc* pooled analysis of the INTERACT and INTERACT2

trials showed that early intervention (<4 h) to reduce blood pressure can improve outcome in haemorrhage (Wang *et al.*, 2015). Although this needs to be examined formally in a prospective trial, it has been noted that clinical management of haemorrhage is already changing as a result of these trials (Rothwell, 2015). Villapol *et al.* found a benefit of sartans after traumatic brain injury when given within 6 h, which is consistent with a neuroprotective effect. Overall, I find it appealing that sartans might improve outcome after traumatic brain injury, and I am sure other groups will now attempt to reproduce and extend these findings. However, preclinical findings have a poor history of replication in many fields including traumatic brain injury, stroke, motor neuron disease and spinal cord injury (Steward *et al.*, 2012).

How can one be certain about sartans? What is the true effect size of any benefit of sartans? Individual studies often serve as poor estimates of the true effect size of an intervention: one typically needs tens of animals per group to achieve high power when effect sizes are small. As has been observed by others, neuroscience experiments in general have low power (Button *et al.*, 2013). It is widely appreciated that underpowered experiments involve a greater risk of false-negative results. However, it is important to note that *P*-values near 0.05 are not reliable unless a study is highly powered (Halsey *et al.*, 2015) and that underpowered experiments also increase the risk of false-positive results (Button *et al.*, 2013).

To be fair to Villapol *et al.*, the number of mice per group in their study is typical for experiments of this kind. Indeed, the authors are to be congratulated for their transparency in reporting this work in detail. The

Experimental Design and Randomisation strategy is well described. The investigator was blinded to treatment during surgery, behavioural observation, and during analysis of some histology. Losses due to mortality during surgery and perfusion are acknowledged and they state that no mice that survived surgery were excluded *post hoc*. Numbers per group are reported clearly in bar charts, which is helpful.

Yet the need to reproduce work independently is well-illustrated in many of the meta-analyses of treatments for neurological injury that have now been published (e.g. CAMARADES; <http://www.dcn.ed.ac.uk/camarades/default.htm>). For example, thousands of animals have been used in tens of well-designed studies to investigate the benefits of hypothermia after stroke (van der Worp *et al.*, 2007). The estimated change in infarct volume or neurological score reported by each study ranges massively, from beneficial to adverse (Fig. 1). Many studies had very large 95% confidence intervals for the estimate of the effect size and many studies found no evidence for a benefit of hypothermia (i.e. those studies had a 95% confidence interval that spanned zero). However, when these heterogeneous studies are put together in a meta-analysis, one obtains an estimate of the effect size which indicates a positive benefit for hypothermia (see grey band in Fig. 1) with a small 95% confidence interval that does not span zero. One can therefore be reasonably certain about hypothermia.

My view is that Villapol *et al.* have performed an interesting study which merits replication and extension. As noted above, one only obtains a precise estimate of the 'true' effect size with a few, larger studies or many, smaller studies. Because sartans are already approved for clinical use,

this is a worthwhile venture. Let's do more to be certain about sartans.

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References

Bath PM, Krishnan K. Interventions for deliberately altering blood pressure in acute

stroke. *Cochrane Database Syst Rev* 2014; 10: CD000039.

Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, et al. Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci* 2013; 14: 365–76.

Halsey LG, Curran-Everett D, Vowler SL, Drummond GB. The fickle P value generates irreproducible results. *Nat Methods* 2015; 12: 179–85.

Rothwell PM. Blood pressure in acute stroke: which questions remain? *Lancet* 2015; 385: 582–5.

Steward O, Popovich PG, Dietrich WD, Kleitman N. Replication and reproducibility in spinal cord injury research. *Exp Neurol* 2012; 233: 597–605.

van der Worp HB, Sena ES, Donnan GA, Howells DW, Macleod MR. Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. *Brain* 2007; 130(Pt 12): 3063–74.

Villapol S, Balarezo MG, Affram K, Saavedra JM, Symes AJ. Neurorestoration after traumatic brain injury through angiotensin II receptor blockage. *Brain* 2015. Advance Access published June 26, 2015.

Wang X, Arima H, Al-Shahi Salman R, Woodward M, Heeley E, Stapf C, et al. Rapid blood pressure lowering according to recovery at different time intervals after acute intracerebral hemorrhage: pooled analysis of the INTERACT studies. *Cerebrovasc Dis* 2015; 39: 242–8.

Does degeneration of the subthalamic nucleus prevent parkinsonism in spinocerebellar ataxia type 2 and type 3?

This scientific commentary refers to 'Why patients with spinocerebellar ataxia type 2 and type 3 do not develop parkinsonism despite severe neuronal loss in the dopaminergic substantia nigra' by Schöls *et al.* (doi:10.1093/brain/awv255).

Spinocerebellar ataxia type 2 (SCA2) and type 3 (SCA3) are autosomal, dominantly inherited progressive ataxia disorders, which are caused by translated CAG repeat expansions that give rise to elongated polyglutamine tracts within ataxin 2 and ataxin 3, respectively. Neurodegeneration in SCA2 and SCA3 mainly affects the cerebellum and brainstem, but other parts of the brain including the substantia nigra are also involved. Nevertheless, typical parkinsonism is only rarely encountered in SCA2 and SCA3. In this issue of *Brain*, Schöls *et al.* (2015) report PET imaging data from 19, and autopsy data from 13, patients with SCA2 or SCA3. Their *in vivo* and post-mortem data confirm severe degeneration of the substantia nigra and add the important finding of a similarly consistent degeneration in the motor territory of the

subthalamic nucleus. Among the autopsied patients, the subthalamic nucleus was spared only in one SCA3 patient who had had parkinsonism during life, suggesting that degeneration of the subthalamic nucleus prevents parkinsonism in SCA2 and SCA3.

The abnormal proteins encoded by the expanded genes in polyglutamine diseases such as SCA2 and SCA3 are ubiquitously expressed in the nervous system. Nevertheless, only certain populations of neurons undergo degeneration, a phenomenon that has been described as selective vulnerability. However, a closer look reveals that neuronal vulnerability in these diseases is by no means selective, but rather is graded. In fact, each polyglutamine disease is characterized by prominent degeneration in certain parts of the nervous system, in SCA2 and SCA3 the cerebellum and brainstem, but clinical, imaging and autopsy findings provide evidence of widespread degeneration involving many parts of the brain and spinal cord. As a rule of thumb, pathology is more extensive with longer CAG repeats. In SCA2 and SCA3, the

dopaminergic substantia nigra is commonly involved. In fact, nigral degeneration was noted in these disorders before the associated gene loci and causative mutations had been discovered. Orozco *et al.* (1989) reported severe loss of substantia nigra neurons in seven autopsied cases of a Cuban ataxia founder population with what later turned out to be SCA2. They also found decreased concentrations of dopamine metabolites in the CSF of patients from this population. Substantia nigra degeneration was noted as a prominent feature of a large American pedigree of Portuguese ancestry published by Rosenberg *et al.* (1976). This paper together with other observations gave rise to the concept of Machado-Joseph disease, a unique multisystem brain degenerative disease, which later became known as SCA3. Severe and early involvement of the dopaminergic nigrostriatal system in SCA3 is underlined by the observation of reduced dopamine transporter density not only in patients, but also in preclinical SCA3 mutation carriers (Yen *et al.*, 2002).