Title: Remote ischaemic conditioning in Carotid Artery Stenting: another step on the journey to towards clinical translatability?

Authors: Robert M Bell and Derek M Yellon

Short title: *RIC in CAS: another step to translation?*

Corresponding author: Derek Yellon

Address: Hatter Cardiovascular Institute,

Institute of Cardiovascular Science

University College London

67 Chenies Mews

London

WC1E 6HX

United Kingdom

Telephone: +44 (0)20 3447 9888

Fax: +44 (0)20 3447 9505

Total Word Count: 2099

Remote ischaemic conditioning is a powerful and highly reproducible cytoprotective intervention demonstrable in a wide range of animal models[1]. To an extent, remote ischaemic conditioning – the phenomena whereby transient, non-lethal ischaemia of a distal tissue bed triggers visceral pro-survival adaption against a lethal ischaemic insult – has also been demonstrable in man, although the necessary landmark clinical outcome study has yet to yield the results necessary for adoption into routine clinical practice.

Remote ischaemic conditioning (RIC) is such a remarkably simple intervention with such a low intrinsic cost in its most simplistic application (manual inflation/deflation of a sphygmomanometer cuff) that it is very attractive to study RIC's efficacy to mitigate ischaemia-related injury in a wide range of pathologies and organ systems. This approach appears to be entirely appropriate: in a variety of experimental settings, remote ischaemic conditioning has proven to be remarkably adept in attenuating ischaemia/reperfusion injury in a variety of organ systems[2], including heart[3], kidney[4], liver[5] and brain[6].

In the *Safety and Efficacy of Remote Ischemic conditioning in patients with Severe carotid Artery Stenosis Prior to Carotid Artery Stenting* study, Ji et al. present an intriguing proof-of-concept trial of RIC to ameliorate the complications of distal thrombo-embolization associated with elective carotid artery stenting (CAS) for severe carotid stenosis. Elective procedures are the ideal setting for ischaemic pre-conditioning (where the conditioning stimulus is applied prior to the anticipated ischaemic insult), and this is the first time remote ischaemic preconditioning has been applied to protect the brain against an embolic complication arising from an elective vascular intervention.

CAS is preferred over carotid endarterectomy (CEA) in patients with high surgical risk, and recent trial data suggest equipoise between the two techniques in intermediate risk patient cohorts and for those selected patients with asymptomatic disease. Thus, in international guidelines[7], CAS has an increasing role in the management of carotid

artery stenosis, but one of the problems associated with the procedure is the risk of thromboembolism and subsequent ischaemic cerebral injury[8]. If a neuroprotective intervention could be brought to bear to mitigate the potential risk, the current equipoise may be tipped in favour of the minimally invasive CAS procedure.

Therefore, it is both welcome and encouraging that Ji et al. report that their remote ischemic conditioning protocol was highly effective in reducing cerebral infarct size following CAS for both severe asymptomatic and symptomatic carotid stenosis.

As a proof-of-concept, the result is exciting, but should of course be taken with some caution: as with our own and others' experience in the context of RIC in cardiac surgery, positive proof-of-concept clinical trials do not inevitably lead to positive clinical outcome studies, as exemplified by the ERICCA[9] and RIPHeart[10].

In this study, looking at the safety and efficacy of RIC in CAS, it is inevitable that the hard clinical outcomes – TIA and stroke – were not significantly different between groups (although the number of events in the RIC group was numerically lower); the trial, by design, was not powered to detect such a difference. Surrogate endpoint analysis, however, revealed a significant benefit in the reduction of DWI lesion volume (0.03ml as compared to sham or control, 0.07 and 0.08ml respectively, $p<0.001$), despite the number of new lesions detected by DWI not being different between groups. This observation would support the anti-ischaemic hypothesis of ischaemic conditioning; the anti-inflammatory hypothesis seemingly not detectable in the measurement of the biomarkers used in the study (hsCRP), nor, oddly, was a detectable change in NSE and S100-b, markers of neurological injury.

Of course, although the results of this study are encouraging, before ischaemic conditioning can be adopted for clinical use, the benefits of the intervention need to be realised in an appropriately powered outcome study. In the case of CAS, one would wish to see that the reduction of DWI volume with RIC be translated into a real-world benefit in terms of stroke, TIA or cognitive preservation and improvement in measures of quality of life.

A commendable feature of this study was the inclusion of a realistic sham control group, where the two upper arm blood pressure cuffs were inflated to 60mmHg. This inflation pressure has the advantage that the patient will be aware of the cuff gaining pressure on the arm, giving the impression of a genuine therapy administration. This is more likely to lead to more effective patient blinding as the patient is much less likely to recognise the sham procedure as such. Importantly, the 60mmHg will not impede arterial flow in the upper limb to trigger tissue ischaemia, although venous flow will be impeded. To counter against the potential for venous stagnation contributing to a RIC-like effect, a second control group was included, where no cuff was applied. Interestingly, there was no observable difference between the sham and the no-intervention control groups. This approach to sham-controlled RIC study design could pave the way for a similar approach to be applied in future sham-control studies: the prior norm for sham interventions has been for lower cuff inflation pressures, typically below the venous occlusion pressure, may be less effective at patient blinding.

This study however raises an important question that all researchers in the field of RIC investigations will be aware. Ji et al. have used a very robust ischaemic conditioning protocol of twice daily, two-arm, five cycle (five minute ischaemia/five minute reperfusion) performed over a period of two weeks prior to surgery. In the majority of prior clinical studies in different clinical settings, the implementation of RIC as preconditioning has been three to four cycles of five minute ischaemia/ five minute reperfusion typically applied to one (very rarely to two) limbs just the once within 40-60 minutes of the index ischaemic event. The RIC protocol in this trial is quite unusual (even compared to the their own post-stroke study, where RIC was applied as conditioning stimulus once daily to two arms over a period of 300 days to prevent recurrent stroke[11]) and one wonders whether this is, in fact, the optimum conditioning protocol to elicit the observed outcome. The problem is that in man, as in animals, there is a remarkable scarcity of data to support any one particular remote ischaemic conditioning protocol over another. In the literature, there has been a creeping increase in the number of cycles of conditioning: a remote ischaemic conditioning “arms” race, with no discernible end in sight. An increased conditioning stimulus may

in part be justified, as research has revealed that cardiovascular morbidities (such as age, hypertension, diabetes[12]) make it harder to trigger cardioprotective-signalling cascades, with comorbidities apparently increasing the threshold of cycles required to trigger protection. Thus, rightly or wrongly, to enable the wide applicability of conditioning, more robust conditioning regimes have been employed to yield a better theoretical outcome. But does more always lead to more? In direct ischaemic conditioning of the organ to be subjected to lethal ischaemia/reperfusion injury, the answer is certainly not: the dose-response curve is "U"-shaped, with greater number of ischaemic cycles and duration leading to a plateau of effectiveness, or even increased injury[13]. Moreover, daily conditioning regimes (for example with pharmacological agonists) don't always lead to optimum protection and perhaps best served by an alternate-day regime[14]. Thus, there is currently no clear, optimal conditioning regimen. To adequately address this problem is clearly a challenge, perhaps one borne out of a lack of a clear biomarker for protection other than the attenuation of ischaemic injury (infarct size) that is typically the primary endpoint of the interventional study. Be it a challenge or not, it is nonetheless incumbent upon researchers to work out the most efficacious conditioning strategy that results in the least discomfiture to the patients that we seek to help.

Positive outcome studies are the key to unlocking the clinical potential of this cheap and easily-applied protective RIC strategy, but the demonstration of a neuroprotective effect of RIC should provide encouragement for further research in this important area of cardiovascular medicine and represents the necessary foundations for future larger outcome-based studies to further optimise patient outcomes in those presenting with severe carotid stenosis.

References:

1. Bromage DI, Pickard JM, Rossello X, Ziff OJ, Burke N, Yellon DM and Davidson SM. Remote ischaemic conditioning reduces infarct size in animal in vivo models of ischaemia-reperfusion injury: a systematic review and meta-analysis. *Cardiovasc Res*. 2016. doi: 10.1093/cvr/cvw219
2. Candilio L, Malik A and Hausenloy DJ. Protection of organs other than the heart by remote ischemic conditioning. *J Cardiovasc Med (Hagerstown)*. 2013;14:193-205. doi: 10.2459/JCM.0b013e328359dd7b
3. Hausenloy DJ and Yellon DM. Ischaemic conditioning and reperfusion injury. *Nature reviews Cardiology*. 2016;13:193-209. doi: 10.1038/nrcardio.2016.5
4. Ho PW, Pang WF and Szeto CC. Remote ischaemic pre-conditioning for the prevention of acute kidney injury. *Nephrology*. 2016;21:274-285. doi: 10.1111/nep.12614
5. Costa FL, Yamaki VN, Goncalves TB, Coelho JV, Percario S and Brito MV. Combined remote ischemic perconditioning and local postconditioning on liver ischemia-reperfusion injury. *J Surg Res*. 2014;192:98-102. doi: 10.1016/j.jss.2014.05.046
6. Hess DC, Blauenfeldt RA, Andersen G, Hougaard KD, Hoda MN, Ding Y and Ji X. Remote ischaemic conditioning-a new paradigm of self-protection in the brain. *Nature reviews Neurology*. 2015;11:698-710. doi: 10.1038/nrneurol.2015.223
7. Brott TG, Halperin JL, Abbara S, Bacharach JM, Barr JD, Bush RL, Cates CU, Creager MA, Fowler SB, Friday G, Hertzberg VS, McIff EB, Moore WS, Panagos PD, Riles TS, Rosenwasser RH, Taylor AJ, Jacobs AK, Smith SC, Jr., Anderson JL, Adams CD, Albert N, Buller CE, Creager MA, Ettinger SM, Guyton RA, Halperin JL, Hochman JS, Hunt SA, Krumholz HM, Kushner FG, Lytle BW, Nishimura RA, Ohman EM, Page RL, Riegel B, Stevenson WG, Tarkington LG and Yancy CW. 2011 ASA/ACCF/AHA/AANN/AANS/ACR/ASNR/CNS/SAIP/SCAI/SIR/SNIS/SVM/SVS guideline on the management of patients with extracranial carotid and vertebral artery disease: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, and the American Stroke Association, American Association of Neuroscience Nurses, American Association of Neurological Surgeons, American

College of Radiology, American Society of Neuroradiology, Congress of Neurological Surgeons, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of NeuroInterventional Surgery, Society for Vascular Medicine, and Society for Vascular Surgery. Developed in collaboration with the American Academy of Neurology and Society of Cardiovascular Computed Tomography. *Catheter Cardiovasc Interv*. 2013;81:E76-E123. doi: 10.1002/ccd.22983

8. Noiphithak R and Liengudom A. Recent Update on Carotid Endarterectomy versus Carotid Artery Stenting. *Cerebrovasc Dis*. 2016;43:68-75. doi: 10.1159/000453282
9. Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, Knight R, Kunst G, Laing C, Nicholas J, Pepper J, Robertson S, Xenou M, Clayton T, Yellon DM and Investigators ET. Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. *N Engl J Med*. 2015;373:1408-1417. doi: 10.1056/Nejmoa1413534
10. Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, Stoppe C, Coburn M, Schaelte G, Boning A, Niemann B, Roesner J, Kletzin F, Strouhal U, Reyher C, Laufenberg-Feldmann R, Ferner M, Brandes IF, Bauer M, Stehr SN, Kortgen A, Wittmann M, Baumgarten G, Meyer-Treschan T, Kienbaum P, Heringlake M, Schon J, Sander M, Treskatsch S, Smul T, Wolwender E, Schilling T, Fuernau G, Hasenclever D, Zacharowski K and Collaborators RIS. A Multicenter Trial of Remote Ischemic Preconditioning for Heart Surgery. *N Engl J Med*. 2015;373:1397-1407. doi: 10.1056/NEJMoa1413579
11. Meng R, Asmaro K, Meng L, Liu Y, Ma C, Xi C, Li G, Ren C, Luo Y, Ling F, Jia J, Hua Y, Wang X, Ding Y, Lo EH and Ji X. Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. *Neurology*. 2012;79:1853-1861. doi: 10.1212/WNL.0b013e318271f76a
12. Bell RM, Botker HE, Carr RD, Davidson SM, Downey JM, Dutka DP, Heusch G, Ibanez B, Macallister R, Stoppe C, Ovize M, Redington A, Walker JM and Yellon DM. 9th Hatter Biannual Meeting: position document on ischaemia/reperfusion injury, conditioning and the ten commandments of cardioprotection. *Basic Res Cardiol*. 2016;111:41. doi: 10.1007/s00395-016-0558-1

13. Johnsen J, Pryds K, Salman R, Lofgren B, Kristiansen SB and Botker HE. The remote ischemic preconditioning algorithm: effect of number of cycles, cycle duration and effector organ mass on efficacy of protection. *Basic Res Cardiol*. 2016;111:10. doi: 10.1007/s00395-016-0529-6
14. Dana A, Baxter GF, Walker JM and Yellon DM. Prolonging the delayed phase of myocardial protection: repetitive adenosine A1 receptor activation maintains rabbit myocardium in a preconditioned state. *J Am Coll Cardiol*. 1998;31:1142-1149. doi: