Potential signalling pathways in human lacunar stroke – A systematic review and meta-analysis of therapeutic strategies in animal models

Protocol of 30/1/2012

Background/Introduction

25% of all ischaemic strokes are of the lacunar subtype, arising from infarcts in the subcortical nuclei of the brain (Sacco et al., 2006). These lacunar infarcts are caused by occlusion of single penetrating arteries which branch from larger arteries around the Circle of Willis. Whilst cardiac embolism is common cause of cortical ischaemic stroke, the Lacunar Hypothesis (Bamford and Warlow, 1988) proposes that key mechanisms in lacunar pathology are lipohyalinosis and microatheroma and that infarcts originating from embolisms are fairly rare. Lipohyalinosis is a small-vessel pathology characterised by abnormal endothelial architecture and fibrosis, whilst microatheroma refers to the accumulation of lipid-containing plaques at the proximal end of penetrating arteries. These can build up in the larger arteries and lead to stenosis or occlusion of the lumen of small penetrating arteries. However, despite associations with endothelial dysfunction and inflammatory disruption of the blood-brain barrier (Knottnerus et al., 2009; Giwa et al., 2012), the causes behind these small vessel changes are poorly understood.

Although lacunar strokes have a relatively good prognosis compared to cortical ischaemic stroke, long-term studies support the view that they are a good indicator of cerebral small vessel pathology which can have severe prognostic implications for recurrent ischaemic strokes (Norrving, 2003). Significant risk factors are hypertension, diabetes, smoking, ageing, and family history of myocardial infarction, as with other types of stroke (Tuszynski et al., 1989). Due to limited understanding of the precise pathological changes which lead to lacunar infarcts, current therapeutic strategies are limited. In addition to this problem, many interventions reported to improve prognosis in animal models of ischaemic stroke fail to show efficacy in humans, suggesting the existence of significant publication bias (Sena et al., 2007), and it is likely the same exists in interventions tested on lacunar stroke models.

In this study I aim to review and analyse the evidence for efficacy of therapeutic strategies tested on animal models of lacunar stroke, and to assess the quality of this evidence, estimating the extend of publication bias in this field.

Objectives

1. To assess the quality of studies investigating therapeutic strategies in animal models of lacunar stroke
2. To assess the efficacy of different therapeutic strategies used to treat animal models of lacunar stroke
3. To identify potential signalling pathways modulated by a therapeutic strategy/therapeutic strategies in animal models of lacunar stroke, comparing these to signalling pathways involving genes associated with risk polymorphisms in humans.

Search strategy

I will carry out an electronic search of Medline, ISI Web of Science and EMBASE using a search strategy previously described by Bailey et al (2009). The search will be carried out for studies published between … and … (DATES), using a search strategy
that will be modified to produce as similar search results as possible depending on the
different functions available for searching each database (Appendix). Search hits will
be downloaded into Reference Manager.

Inclusion Criteria
Types of studies – Experiments comparing a suitable control which describe the effect
of an intervention in animal models of lacunar stroke, or those in which small
subcortical lesions in white or gray matter are produced.
Types of animals – In vivo animal experiments
Types of intervention – Administration of any intervention aimed at improving outcome
and reducing lacunar stroke pathology
Types of outcome measures – Brain examination: histology, molecular analyses,
behavioural outcomes

Exclusion Criteria
All lesions greater than 1/140 of total brain volume to allow infarcts to be proportional
to animal brain size – lesions greater than 100cm3 in 1400cm3 human brain were
excluded in Bailey et al (2009)
Middle cerebral artery occlusions which produce only cortical infarcts
Human studies.

Methods
Assessment of quality – Study quality will be rated against the 10 items on the
CAMARADES checklist (Sena et al, 2007):
1. Publication in peer reviewed journal
2. Statement of control of temperature
3. Randomisation of treatment or control
4. Allocation concealment
5. Blinded assessment of outcome
6. Avoidance of anaesthetics with marked intrinsic neuroprotective properties¹
7. Use of animals with relevant co-morbidities
8. Statement of sample size calculation
9. Statement of compliance with regulatory requirements
10. Statement regarding possible conflicts of interest

Data Extraction – I will investigate comparisons between the outcome of groups of
animals receiving a specified dose(s) at a specific time(s) and the outcome of control
groups. Other data extracted will include: author, year of publication, intervention used,
animal model used (including species, strain and sex), number of animals in each
group, standard deviation and/or standard error, presence of multiple interventions in
one treatment group, presence of salt loading, method of stroke induction, anaesthetic
used, time and type of outcome measurement², and time of intervention administration.
Outcome measurements will be recorded at the latest time of assessment. In cases
where only graphical data can be found, I will attempt to contact the author. If this is
not possible I will estimate values by direct measurement from the graphs provided in
the publications.

¹ Revision July 2012: Many of the studies identified do not use anaesthesia, so this quality item was removed
² Revision July 2012: For studies using the stroke prone SHR these times were not available and so analyses of
these variables will exclude SHRSP
Analysis – Data will be analysed using random effects meta-analysis performed within the CAMRADES Microsoft Access database, using normalised mean differences and standardised mean differences where appropriate. In cases where a single control group serves multiple treatment groups, the size of the control group used for the meta-analysis will be divided by the total number of treatment groups it serves. Data will be used to conduct a stratified meta-analysis to identify any significant contribution of individual study characteristics or study quality items on effect size. Meta-regression will be also used where appropriate. P-values will be adjusted using the Bonferroni correction to evaluate significant results.

References

Revised October 2013: In light of reviewer’s comments on submitted manuscript and to improve the clarity of the analysis of the impact of randomisation and blinding we will in addition calculate an effect size for the impact of non-reporting of these risk of bias items, and 95% confidence intervals for these. This effect size will be expressed as a percentage change in efficacy compared with studies which do report randomisation or blinding; and variance of this effect size will be calculated as: 

\[
\sqrt{\left(\frac{n_1 + n_2}{n_1 \cdot n_2}\right) \times \left(\frac{\frac{(n_3 \cdot (n_1 - 1) \cdot se^2_1) + (n_2 \cdot (n_2 - 1) \cdot se^2_2)}{n_1 + n_2 - 1}}{n_1 + n_2 - 1}\right)}
\]