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Preventing cognitive decline and dementia from cerebral small vessel disease: The LACI-1 Trial. Protocol and statistical analysis plan of a phase IIa dose escalation trial testing tolerability, safety and effect on intermediary endpoints of isosorbide mononitrate and cilostazol, separately and in combination

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Abstract
Rationale: The pathophysiology of most lacunar stroke, a form of small vessel disease, is thought to differ from large artery atherothrombo- or cardio-embolic stroke. Licensed drugs, isosorbide mononitrate and cilostazol, have promising mechanisms of action to support their testing to prevent stroke recurrence, cognitive impairment, or radiological progression after lacunar stroke.

Aim: LACI-I will assess the tolerability, safety, and efficacy, by dose, of isosorbide mononitrate and cilostazol, alone and in combination, in patients with ischemic lacunar stroke.

Sample size: A sample of 60 provides 80% power (significance 0.05) to detect a difference of 35% (90% versus 55%) between those reaching target dose on one versus both drugs.

Methods and design: LACI-I is a phase IIa partial factorial, dose-escalation, prospective, randomized, open label, blinded endpoint trial. Participants are randomized to isosorbide mononitrate and/or cilostazol for 11 weeks with dose escalation to target as tolerated in two centers (Edinburgh, Nottingham). At three visits, tolerability, safety, blood pressure, pulse wave velocity, and platelet function are assessed, plus magnetic resonance imaging to assess cerebrovascular reactivity in a subgroup.

Study outcomes: Primary: proportion of patients completing study achieving target maximum dose.

Secondary: symptoms whilst taking medications; safety (hemorrhage, recurrent vascular events, falls); blood pressure, platelet function, arterial stiffness, and cerebrovascular reactivity.

Discussion: This study will inform the design of a larger phase III trial of isosorbide mononitrate and cilostazol in lacunar stroke, whilst providing data on the drugs' effects on vascular and platelet function.

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Introduction and rationale

Cerebral small vessel disease (SVD) is a common disorder that affects small perforating arterioles in the brain’s deep white and gray matter. It causes 25% of ischemic strokes (“lacunar” stroke), intracerebral hemorrhage, vascular and many mixed dementias, gait and bladder dysfunction. Cardiovascular and atherosclerotic disease are uncommon in lacunar stroke and SVD, respectively. Although the pathophysiology of SVD remains poorly understood, endothelial dysfunction, inflammation, blood–brain barrier failure, and impaired vasoreactivity have been demonstrated. There is no specific secondary prevention for lacunar stroke, SVD-associated dementia, or progression of SVD lesions on neuroimaging. We recently summarized available drugs with potentially relevant actions and identified two agents that seemed worthy of further testing: isosorbide mononitrate (ISMN) and cilostazol.

ISMN, a nitric oxide (NO) donor, is commonly used in angina. NO levels are reduced in acute, chronic and possibly in lacunar stroke. NO has many potentially beneficial effects for SVD including improved vasoreactivity, neuroprotection, and anti-inflammatory effects. In the Efficacy of Nitric Oxide in Stroke (ENOS) trial, the NO donor glyceryl trinitrate administered within 6 h of all types of stroke, improved cognitive test scores at 90 days. However, there are few data on ISMN in lacunar stroke in part because ischemic heart disease, for which ISMN is licensed, is relatively infrequent in those with SVD.

Cilostazol is a phosphodiesterase 3 inhibitor, mainly used for peripheral vascular disease in Europe and North America, but more widely used for cerebrovascular disease prevention in Asia-Pacific countries. Cilostazol has mild antiplatelet effects plus several potentially beneficial effects for SVD including improved blood–brain barrier integrity, vasodilatory and anti-proliferative activity, improves oligodendrocyte maturation and hence myelination, and reduces white cell chemotaxis. In models, it improved motor and cognitive function, and reduced infarct volume and in human lacunar stroke it improved middle cerebral artery pulsatility index. Over 6000 patients, many with lacunar stroke, have been included in trials of cilostazol in secondary stroke prevention mostly in Asia-Pacific countries (Figure 1); a meta-analysis of these suggested that cilostazol reduces recurrent stroke.

There is little experience of ISMN in lacunar stroke, cilostazol is rarely used for stroke prevention in Europe or the Americas. There are no data on the effects of cilostazol when combined with ISMN yet the effects are potentially synergistic.

Therefore, the Lacunar Intervention Trial-1 (LACI-1) will test ISMN and cilostazol, alone and combined, in patients with lacunar ischemic stroke, to assess their tolerability, safety, and efficacy on mechanistic endpoints including cerebrovascular reactivity assessed using magnetic resonance imaging (MRI). Treatment will be given in addition to current guideline-based post-stroke secondary prevention. LACI-1 will inform the design of a larger trial to test ISMN and cilostazol effects on recurrent vascular events, cognition, disability, death, and SVD lesion progression on MRI (LACI-2). LACI-1 was designed through a UK National Institute for Health Research Stroke Research Network Expert Writing Group.

Methods

Design

Phase IIa, partial factorial, dose-escalation, prospective, randomized, open label, blinded endpoint (PROBE) trial conducted in two UK centers (Edinburgh, Nottingham).

Participants are randomized in a 1:1:1:1 ratio into four groups: cilostazol alone; ISMN alone; cilostazol and ISMN combined, started immediately (with ISMN given first); and cilostazol and ISMN combined, start delayed for three weeks (cilostazol first) (Figure 2). The delayed start group also provides a “no drug” comparison group during the first three weeks.

Participants take trial medication for 11 weeks. The dose is increased, in weekly increments over two to three weeks as tolerated, sustained until eight weeks post-randomization, then decreased gradually over two weeks and stopped (Supplementary Information...
gives medication by study week). The escalating dose is designed to reduce potential adverse effects following initiation of cilostazol and is standard for ISMN. Gradual dose reduction aims to prevent large hemodynamic changes on cessation of medication.

**Patient population**

**Inclusion:**

1. Mild symptomatic ischemic lacunar stroke in the past four years, compatible with a clinical lacunar stroke syndrome, with brain MRI or CT scanning showing a symptomatic small subcortical (lacunar) infarct (<20 mm), or if no recent relevant infarct is visible, that excluded other cause for symptoms. Clinical or imaging evidence of a prior non-lacunar stroke is not an exclusion as long as the randomizing clinician is confident that the non-lacunar stroke is not responsible for the index lacunar stroke symptoms.
2. Age ≥ 35 years.
3. Independent in activities of daily living (modified Rankin Scale of ≤ 2) and able to give informed consent.

**Exclusion:**

1. Other significant neurological illness since the incident stroke.
2. Age < 35 years.
4. Requiring assistance with activities of daily living (modified Rankin Scale ≥ 3).
5. Active cardiac disease.
6. Carotid stenosis > 50% (NASCET criteria) on the side of the symptomatic stroke lesion requiring urgent intervention. Note: successfully treated carotid artery stenosis may be included.
7. Definite indication for, or contraindication to, cilostazol or ISMN.
8. Unable to swallow.
10. Unlikely to comply with trial medication based on past history or lifestyle.
11. Planned surgery during the trial period.
12. History of intracranial hemorrhage (but not asymptomatic hemorrhagic transformation of an infarct).
13. Other life threatening illness.
14. History of drug overdose, attempted suicide or significant active mental illness.
15. Pregnant or breastfeeding women.
17. Use of prohibited medications (anticoagulants, phosphodiesterase 5 inhibitors, macrolides, ketoconazole, itraconazole, omeprazole).
20. Current enrolment in another Clinical Trial of Investigational Medicinal Product (CTIMP).

**Randomization**

Baseline information is entered on a password-protected website (https://nottingham.ac.uk/~nszwww/prev-svd/). Once checked and complete, a computer algorithm randomizes participants at a 1:1:1:1 ratio to a study group. Randomization is minimized on age ≤/≥ 70 years, SVD severity on brain scanning (SVD score ≤/≥ 2), systolic blood pressure ≤/≥ 140 mmHg and time after stroke ≤/≥ 100 days.
Figure 2. Flow chart of study procedures.

1. **Identify eligible patients**
   - Consent
   - **Visit 1 Baseline**
     - Check eligibility
     - Medical history
     - Diagnostic MRI or CT assessment
     - Cognitive testing
     - Cerebrovascular reactivity MRI (Edinburgh patients only)
   - **Randomisation**

2. **Week 1**
   - **Group 1**
     - Isosorbide mononitrate alone
     - Start immediately
   - **Group 2**
     - Cilostazol alone
     - Start immediately
   - **Group 3**
     - Isosorbide mononitrate and cilostazol
     - Start immediately
     - ISMN first
   - **Group 4**
     - Isosorbide mononitrate and cilostazol
     - Delayed start
     - Cilostazol first

   - **End of Week 1 phone call follow-up**
     - Structured questionnaire about symptoms and tablet compliance

3. **End of Week 2 phone call follow-up**
   - Structured questionnaire about symptoms and tablet compliance
   - **Visit 2 (Week 3)**
     - Structured questionnaire about symptoms and tablet compliance
   - Blood pressure
   - Platelet test
   - PWV

4. **End of Week 5 phone call follow-up**
   - Structured questionnaire about symptoms and tablet compliance

5. **End of Week 7 phone call follow-up**
   - Structured questionnaire about symptoms and tablet compliance
   - **Visit 3 (Week 8)**
     - Structured questionnaire about symptoms and tablet compliance
     - Cognitive testing
   - Blood pressure
   - Platelet test
   - PWV

6. **Decrease tablets**
7. **Week 11 phone call follow-up**
   - Structured questionnaire about symptoms and tablet compliance
**Intervention**

The starting dose for cilostazol is 50 mg twice daily, increasing to 100 mg twice daily (target dose). The starting dose of ISMN is 25 mg once daily increasing to 25 mg twice daily (target dose). Participants allocated to both drugs aim to attain the same target doses as for the drugs alone (dose schedules in Supplementary Information). Unused tablets are returned to Pharmacy for counting and destruction at the end of the 11-week period.

**Primary outcome**

The proportion of participants achieving target dose assessed by alternate weeks structured questionnaire, supplemented by diary and Pharmacy tablet count.

**Secondary outcomes**

1. Symptoms (headache, nausea, diarrhea, vomiting, bleeding) recorded by structured questionnaire.
2. Safety (systemic or intracranial bleeding, recurrent vascular events, death).
4. Platelet function (P-selectin flow cytometry).
5. Systemic arterial stiffness (pulse wave velocity and pulse wave analysis using the SphygmoCor tonometry device).
6. In a subgroup recruited in Edinburgh, cerebrovascular reactivity (CVR) in white matter and cerebrospinal fluid and blood pulsatility, assessed using MRI. Acquisition details are provided in the Supplementary Information.

**Blinding**

The processing and analysis of CVR, platelet function, pulse wave velocity, blood pressure, and all tablet counts, study questionnaires and compliance data will be performed blind to treatment allocation. Apart from the study research fellow and research nurse, other staff performing the above assessments will not be aware of the treatment allocation, particularly during image analysis, blood tests and follow-up data analysis. When talking to participants, the tablets will only be referred to as “A” or “B” to facilitate patients’ understanding of procedures.

**Data monitoring committee**

An independent data monitoring committee (DMC) is established, chair Prof. Colin Baigent (Oxford).

**Sample size**

A sample size of 55 provides 80% power (significance 0.05) to detect a difference of 90% versus 55% (i.e. an absolute difference of 35%) between those reaching target doses on one drug versus both drugs; i.e. we expect that 35% fewer patients will tolerate both versus one of the two drugs and the sample size is set to be able to detect that difference. For CVR, lacunar stroke patients have impaired middle cerebral artery vasoreactivity on transcranial Doppler ultrasound with an effect size of 25%, standard deviation (SD) of 40%. Little data exist on CVR measured by blood oxygen level dependent (BOLD) MRI in lacunar stroke. The 40-participant sample in Edinburgh will allow detection of a relative difference in CVR of 25% (4% versus 3% signal; with estimated common SD of 40%) between no treatment and target dose of both drugs, significance 0.05 and power 0.80. Allowing for losses, 60 participants will be recruited in total.

**Analyses**

**Image processing.** Structural MR images will be scored for SVD lesion burden using validated scales and processed to generate tissue segmentation maps using validated software. CVR (% signal change/mmHg change in end-tidal CO₂) will be determined by multiple linear regression of the BOLD MRI signal time course, with the end-tidal CO₂ and time (to account for scanner drift) as regressors, for specific tissue regions for comparison of trial drugs and doses.

**Statistical.** We will compare cilostazol versus none, ISMN versus none, and cilostazol and ISMN given immediately versus both given after a delay (the delayed start provides a drug-free control period; having two groups that both get both drugs compares drug initiation with one versus the other drug). We will compare symptoms, blood pressure, arterial stiffness, platelet function, and CVR by treatment allocation. The primary outcome (proportion of participants achieving target dose) will be assessed using binary logistic regression with adjustment for minimization factors; age, SVD score, systolic blood pressure, and time from stroke to randomization (days). The secondary outcomes will be assessed using binary logistic regression for binary variables, multiple linear regression for continuous variables and Cox proportional hazards regression for variables which have a time- until-event component. As data are collected over an 11-week period, time trends will also be examined. Data tables and figures summarizing the main planned comparisons are provided in the Supplementary Information.
Study organization and funding

The study is funded by The Alzheimer’s Society and will be performed in the Centre for Clinical Brain Sciences and Edinburgh Clinical Trials Unit, University of Edinburgh and the Stroke Trials Unit, Division of Clinical Neuroscience, University of Nottingham.

Discussion

Intensive assessment

LACI-1 follows participants intensively over 11 weeks including 3 research clinic visits (to assess BP, platelet function, arterial stiffness, CVR, and symptoms) and 5 telephone follow-ups (to assess side-effects and guide dose escalation). The intense follow-up provides data on dose escalation and safety to inform a future larger, pragmatic, phase III trial with less frequent follow-up.

PROBE design

The PROBE design blinds the main study outcomes whilst maintaining feasibility of dose escalation. A double-blind design proved to be impractical as there is no matching placebo for either study drug. Furthermore, masking by over-encapsulation was impractical and prohibitively expensive when combined with dose escalation. Complicated arrangements for dispensing multiple bottles of study drug, with different dose combinations, were required, with high risk of reduced compliance, confusion, and incorrect medication. The PROBE design is well established and the
study has been designed and staffed appropriately to maintain investigator blinding at the point of outcome assessment.

**Factorial design**

The factorial design compares each drug to no drug and also combination therapy to no therapy. The “delayed start” group provides a modified “no-drug” control group as these participants do not receive medication for the first three weeks after randomization so are not on any medication at the second visit (week 3). They start medication following the week 3 visit, creating a more efficient design, as all participants receive study drug and tests the effect of which drug is commenced first in the combination groups (Supplementary Information). Randomization using minimization increases statistical power.

**Dose escalation**

The common side-effects of both study drugs (ISMN: headache; cilostazol: headache, palpitations) are usually encountered soon after starting the medication. Slow dose escalation at treatment initiation is widely used to lessen these side-effects. However, evidence for this comes from a single non-randomized study and personal experience. Dose escalation of dipyridamole, a phosphodiesterase 5 inhibitor with a similar pharmacodynamics profile to cilostazol, did not reduce headache in a blinded randomized comparison. LACI-1 will provide objective evidence on the frequency of common inception side-effects to inform a larger pragmatic trial at up to 20 sites to select the regimen that best balances simplicity with tolerability and compliance.

**Mechanistic endpoints**

Stroke recurrence, whilst a significant problem following lacunar stroke, occurs relatively infrequently and late, whilst radiological progression of SVD is relatively slow. Hence large trials with long follow-up periods are required to detect treatment effects. To enhance information on pharmacological effects of cilostazol and ISMN at these doses, we will use mechanistic vascular function endpoints (Figure 3): changes in CVR measured using hypercapnic challenge BOLD MRI scans, and changes in arterial, venous, and cerebrospinal fluid flow characteristics measured using phase-contrast MRI; improvement in systemic vascular stiffness using pulse wave velocity (SphygmoCor tonometry device); and alteration in platelet function will test effects on platelet activation and provide safety data.

**Bleeding**

Cilostazol (but not ISMN) has a low risk of bleeding, a potential interaction with the background antiplatelet medication that patients will be taking. One of the aims of LACI-1 is to assess whether bleeding is enhanced when patients take these combinations of antiplatelet agents.

**Summary and conclusions**

LACI-1 will provide data on tolerability, safety and surrogate efficacy markers for cilostazol and ISMN in patients with lacunar stroke, and will inform the design of a larger pragmatic phase III study.

**Declaration of conflicting interests**

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**Authors’ contributions**

Gordon W Blair participated in study design, study set-up, manuscript preparation, and figures. Jason P Appleton participated in study design, study set-up, and manuscript preparation. Zhe Kang Law contributed in study set-up and supervision. Fergus Doubl contributed study design, supervision, study set-up and manuscript preparation. Katie Flaherty contributed for study design, statistics and manuscript preparation. Richard Dooley contributed for study design, randomization algorithm and manuscript
preparation. Kirsten Shuler and Carla Richardson participated in trial management, study set-up and manuscript preparation. Iona Hamilton contributed for study set-up, imaging protocol and manuscript preparation. Yulu Shi and Michael Stringer participated in study set-up and manuscript preparation. Julia Boyd has done the trial management and manuscript preparation. Michael J Thrippleton has participated in manuscript preparation, study design, and imaging protocol. Nikola Sprigg designed the study and supervised the manuscript preparation. Philip M Bath contributed for conception, study design, supervision, and manuscript preparation. Joanna M Wardlaw contributed for conception, funding, study design, supervision, and manuscript preparation.

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