Title: 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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Main text

Supplementary Information

Abstract:

Rationale
The pathophysiology of most lacunar stroke, a form of small vessel disease (SVD), is thought to differ from large artery atherothrombo- or cardio-embolic stroke. Licenced drugs, isosorbide mononitrate (ISMN) 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-1 will assess the tolerability, safety and efficacy, by dose, of ISMN and cilostazol, alone and in combination, in patients with ischaemic 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-1 is a phase IIa partial factorial, dose-escalation, prospective, randomised, open label, blinded endpoint (PROBE) trial. Participants are randomised to ISMN and/or cilostazol for 11 weeks with dose escalation to target as tolerated in two centres (Edinburgh, Nottingham). At three visits, tolerability, safety, blood pressure, pulse wave velocity and platelet function are assessed, plus magnetic resonance imaging (MRI) 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 (haemorrhage, 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 ISMN and cilostazol in lacunar stroke, whilst providing data on the drugs' effects on vascular and platelet function.

Trial Registration: ISRCTN (ISRCTN12580546) and EudraCT (2015-001953-33)

Introduction and rationale

Cerebral small vessel disease (SVD) is a common disorder that affects small perforating arterioles in the brain's deep white and grey matter.(1) It causes 25% of ischaemic strokes ('lacunar' stroke), intracerebral haemorrhage, vascular and many mixed dementias, gait and bladder dysfunction(1, 2). Cardioembolism and atherothromboembolism are uncommon in lacunar stroke and SVD. Although the pathophysiology remains poorly understood, endothelial dysfunction(1, 3, 4), inflammation(3, 5), blood-brain barrier failure(6) and impaired vasoreactivity(4, 7) have been demonstrated.

There is no specific secondary prevention for lacunar stroke, SVD-associated dementia or progression of SVD lesions on neuroimaging(1). We recently summarised available drugs with potentially relevant actions and identified two agents that seemed worthy of further testing: isosorbide mononitrate (ISMN) and cilostazol(8).

ISMN, a nitric oxide (NO) donor, is commonly used in angina. NO levels are reduced in acute,(9) chronic (10) and possibly in lacunar stroke(11). NO has many potentially beneficial effects for SVD including improved vasoreactivity, neuroprotection and anti-inflammatory effects(8). In the Efficacy of Nitric Oxide in Stroke (ENOS) trial, the NO donor glyceryl trinitrate administered within six hours of all types of stroke, improved cognitive test scores at 90 days (12). However, there are few data on ISMN in lacunar stroke in part because ischaemic heart disease, for which ISMN is licensed, is relatively infrequent in those with SVD(13).

Cilostazol is a phosphodiesterase 3' inhibitor(8), mainly used for peripheral vascular disease in Europe and North America(14), 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(15), and reduces white cell chemotaxis(8). In models it improved motor and cognitive function, and reduced infarct volume(16) and in human lacunar stroke it improved middle cerebral artery pulsatility index(17). Over 6000 patients, many with lacunar stroke, have been included in trials of cilostazol in secondary stroke prevention mostly in Asia-Pacific countries (Fig. 1); a meta-analysis of these suggested that cilostazol reduces recurrent stroke(18-21).

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(8).

Therefore, the Lacunar Intervention Trial-1 (LACI-1) will test ISMN and cilostazol, alone and combined, in patients with lacunar ischaemic 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, randomised, open label, blinded endpoint (PROBE) trial conducted in two UK centres (Edinburgh, Nottingham).

Participants are randomised 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) (Fig. 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 2-3 weeks as tolerated, sustained until 8 weeks post-randomisation, 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 haemodynamic changes on cessation of medication.

Patient population

Inclusion:

1. Mild symptomatic ischaemic 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 (<20mm), 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 randomising 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
3. Montreal Cognitive Assessment (MoCA) <20
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
9. Bleeding tendency
10. Unlikely to comply with trial medication based on past history or lifestyle
11. Planned surgery during the trial period
12. History of intracranial haemorrhage (but not asymptomatic haemorrhagic 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
16. Women of childbearing age not taking contraception
17. Use of prohibited medications (anticoagulants, phosphodiesterase 5' inhibitors, macrolides, ketoconazole, itraconazole, omeprazole)
18. Creatinine clearance < 25ml/min
19. Hepatic impairment
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 randomises participants at a 1:1:1:1 ratio to a study group. Randomisation is minimised on age ≤/>70 years, SVD severity on brain scanning (SVD score ≤/>2)(22), systolic blood pressure ≤/>140mmHg and time after stroke ≤/>100 days.

Intervention

The starting dose for cilostazol is 50mg twice daily, increasing to 100mg twice daily (target dose). The starting dose of ISMN is 25mg once daily increasing to 25mg 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, diarrhoea, vomiting, bleeding) recorded by structured questionnaire.

2) Safety (systemic or intracranial bleeding, recurrent vascular events, death).

3) Blood pressure.

4) Platelet function (P-selectin flow cytometry)(23)

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)(7) 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% (ie an absolute difference of 35%) between those reaching target doses on one drug versus both drugs; ie 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% (4). Little data exist on CVR measured by blood oxygen level dependent (BOLD) MRI in lacunar stroke(7). The 40 participant sample in Edinburgh will allow detection of a relative difference in CVR of 25% (4% vs 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(24) and processed to generate tissue segmentation maps using validated software(25). CVR (% signal change/mmHg CO_2) will be determined by multiple linear regression of the BOLD MRI signal time course, with the end-tidal CO_2 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 vs. none, ISMN vs. none, and cilostazol and ISMN given immediately vs. 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 vs. 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 minimisation factors; age, SVD score, systolic blood pressure and time from stroke to randomisation (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 eleven- week period, time trends will also be examined.

Data tables and figures summarising 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 three research clinic visits (to assess BP, platelet function, arterial stiffness, CVR and symptoms) and five 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(26) 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 randomisation 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). Randomisation using minimisation 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-randomised study(27) 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 randomised comparison(28). 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(29). 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 (Fig. 3): changes in CVR measured using hypercapnic challenge BOLD MRI scans(7), 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 (30).

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.

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Author Contributions

Gordon W Blair –study design, study set up, manuscript preparation, figures

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Zhe Kang Law - study set up, preparation

Fergus Doubal -,study design, supervision, study set up, manuscript preparation

Katie Flaherty-, study design, statistics, manuscript preparation

Richard Dooley-, study design, randomisation algorithm, manuscript preparation

Kirsten Shuler- trial management, study set up, manuscript preparation,

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Michael J Thrippleton- manuscript preparation, study design, imaging protocol

Nikola Sprigg- study design,,supervision, manuscript preparation,

Philip M Bath- conception, study design, supervision, manuscript preparation,

Joanna M Wardlaw- conception, funding, study design, supervision, manuscript preparation

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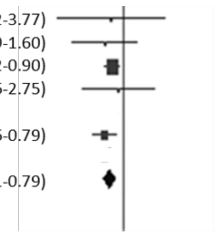
Figures

Figure 1. Meta-analysis of trials of cilostazol for secondary stroke prevention(31-35).

Study	Lacunar proportion	Cilostazol group events	Cilostazol group size	Control group events	Control group size	Weight	Odds Ratio	95% Confidence Interval
Nakamura[31]	47%	2	38	3	38	1.3%	0.67	(0.12-3.77)
CAIST[32]	55%	5	231	9	227	3.4%	0.55	(0.19-1.60)
CSPS 2[33]	65%	82	1337	119	1335	54.0%	0.69	(0.52-0.90)
TOHOKU (Shimizu)[34]	67%	5	251	6	256	2.9%	0.85	(0.26-2.75)
CSPS[35]	75%	34	533	64	534	25.0%	0.53	(0.36-0.79)
Total		128	2390	201	2390		0.64	(0.51-0.79)



Heterogeneity: Tau2=0.00;Chi2=1.41, df=4(P=0.84);I2=0%
Test for overall effect: Z=4.13(P<0.0001)

Figure 2: Flow chart of study procedures

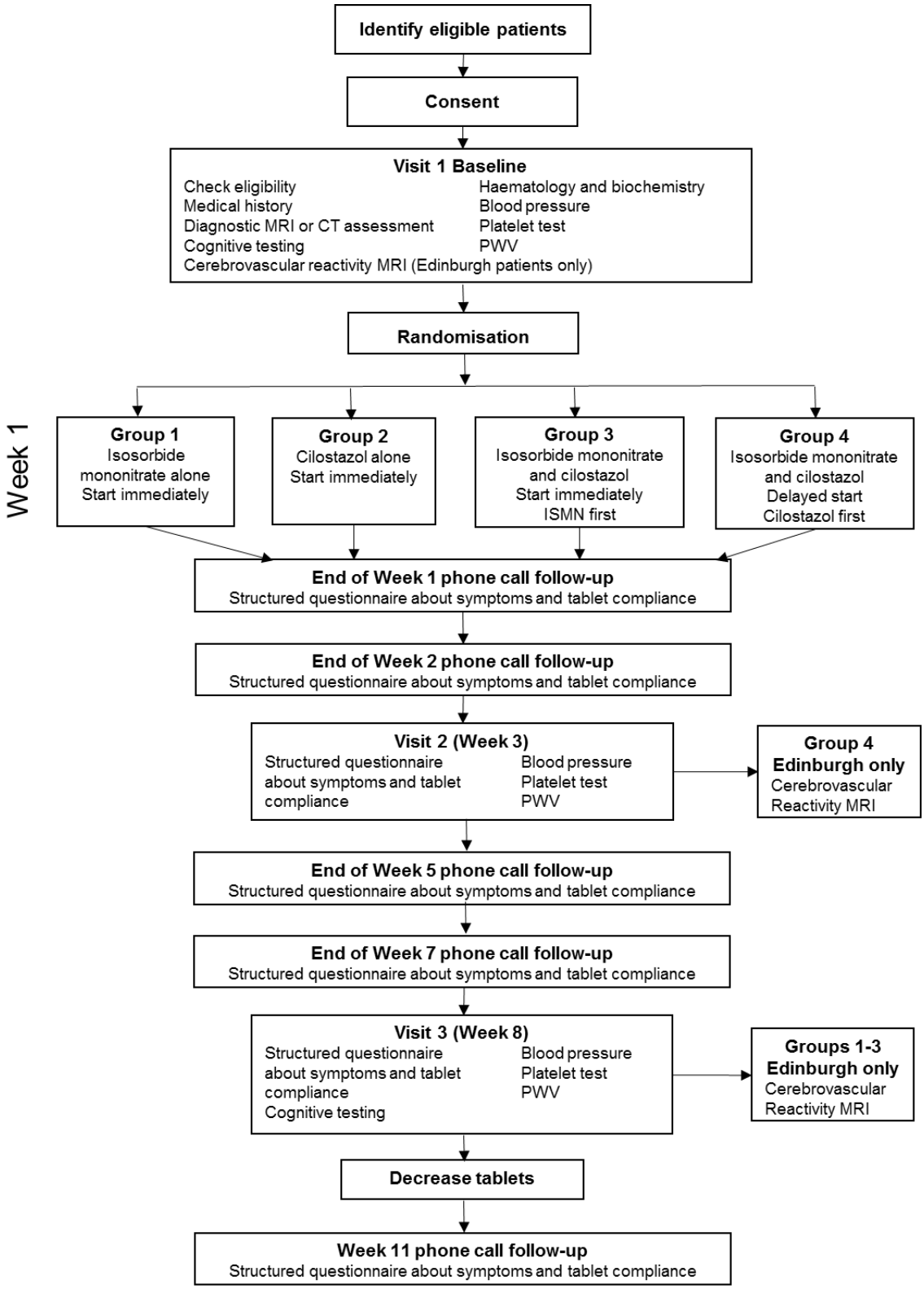
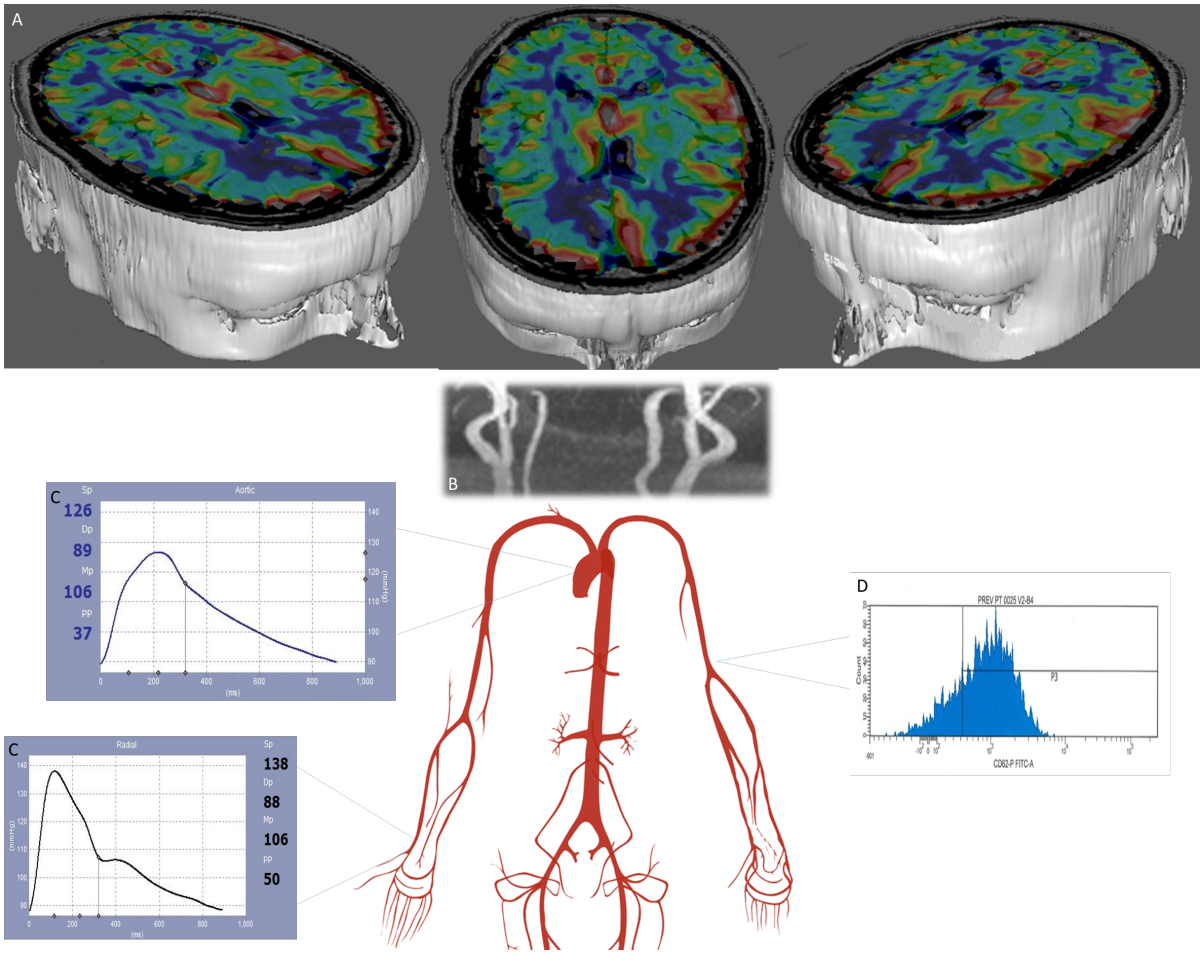


Figure 3: Mechanistic endpoints (‘intermediary outcomes’) assessing brain and systemic vascular function in response to cilostazol and isosorbide mononitrate. A) Brain – CVR MRI scans. The dark blue areas indicate the least reactive tissues, the bright red areas are the most reactive tissues. B) Carotid and vertebral arteries – phase contrast MRI scan, which also measures intracranial arterial, venous sinus and CSF flow. C) Systemic arteries -Aortic and Radial artery pressure waveforms and pulse wave velocity. D) Platelet function is assessed with P-Selectin flow cytometry.



Supplementary Information

List of Supplementary Information

S1 Drug dosing tables

S2 Statistical analysis tables 1 - 8

S3 Imaging protocol

S1 Drug dosing tables

Group 1: Patients randomised to Isosorbide Mononitrate alone

Group 1 Dosing Regime		
Week	ISMN	
	Morning	Evening
1	Nil	25mg
2	25mg	25mg
3	25mg	25mg
4	25mg	25mg
5	25mg	25mg
6	25mg	25mg
7	25mg	25mg
8	25mg	25mg
9	25mg	Nil
10	Nil	Nil
11	Nil	Nil

Group 2: Patients randomised to cilostazol alone

Group 2 Dosing Regime		
Week	Cilostazol	
	Morning	Evening
1	50mg	50mg
2	100mg	50mg
3	100mg	100mg
4	100mg	100mg
5	100mg	100mg
6	100mg	100mg
7	100mg	100mg
8	100mg	100mg
9	50mg	50mg
10	Nil	Nil
11	Nil	Nil

Group 3: Patients randomised to both Isosorbide Mononitrate and Cilostazol - start immediately – ISMN first

Group 3 Dosing Regime				
Week	**Isosorbide Mononitrate**		**Cilostazol**	
	Morning	**Evening**	**Morning**	**Evening**
1	Nil	25mg	Nil	Nil
2	25mg	25mg	Nil	Nil
3	25mg	25mg	50mg	50mg
4	25mg	25mg	100mg	50mg
5	25mg	25mg	100mg	100mg
6	25mg	25mg	100mg	100mg
7	25mg	25mg	100mg	100mg
8	25mg	25mg	100mg	100mg
9	25mg	Nil	50mg	50mg
10	Nil	Nil	Nil	Nil
11	Nil	Nil	Nil	Nil

Group 4: Patients randomised to both Isosorbide Mononitrate and Cilostazol - delayed start – cilostazol first - Group 4:

Group 4 Dosing Regime				
Week	**Isosorbide Mononitrate**		**Cilostazol**	
	Morning	**Evening**	**Morning**	**Evening**
1	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil
4	Nil	Nil	50mg	50mg
5	Nil	Nil	100mg	50mg
6	Nil	25mg	100mg	100mg
7	25mg	25mg	100mg	100mg
8	25mg	25mg	100mg	100mg
9	25mg	Nil	50mg	50mg
10	Nil	Nil	Nil	Nil
11	Nil	Nil	Nil	Nil

S2 Data tables for statistical analysis

Table 1. Baseline characteristics. Data are number (%), median [interquartile range] or mean (standard deviation).

Variable	All	Both delayed	ISMN	Cilostazol	Both immediate
Patients (%)					
Age (years)					
Sex, female (%)					
Onset to randomisation (days)					
Scan type, MRI (%)					
Visible acute stroke lesion MRI (%)					
Lesion visible on DWI (%)					
Lesion visible on FLAIR (%)					
Side of brain, left (%)					
SVD score MRI (/4)					
≥1 Lacune (%)					
≥1 Microbleeds (%)					
Perivascular spaces (%)					
WMH Fazekas MRI					
Visible acute stroke lesion CT (%)					
Side of brain left (%)					
SVD score CT (/2					
≥1 Lacune (%)					
WMH Score					
Atrophy					
Systolic BP (mmHg)					
Diastolic BP (mmHg)					
Arm used for BP, left (%)					
Past medical history (%)					
Treated hypertension					
Treated hyperlipidaemia					
Diabetes					
Atrial fibrillation					
Ipsilateral carotid stenosis >50%					
Peripheral arterial disease					
Heart failure					
Angina					
Myocardial infarction					
Previous stroke					
Previous TIA					
Stroke in immediate family					
Smoking					
Alcohol intake [upw]					
Additional salt (%)					
mRS – pre-stroke [/6]					
mRS – baseline [/6]					
Current NIHSS					
MoCA (/30)					
TMT Part A (secs)					
TMT test Part A (points)					
TMT Part B – (secs)					
TMT Part B – (points)					
National adult reading test (error score)					

mRs, modified Rankin Score; NIHSS, National Institutes of Health Stroke Scale; MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test.

S2, **Table 2.** Tablet compliance. Note, here 'full dose' is defined as achieving the whole dose prescribed for that time period (e.g. 25mg/day for week 1 of ISMN); 'partial dose' is defined as achieving part of the dose prescribed for that time period (e.g. 25mg only on alternate days for ISMN).

Variable	All	Both delayed	ISMN	Cilostazol	Both immediate
Week 1 (telephone)					
Adherence full dose (%)					
Adherence partial dose (%)					
No. doses missed					
Week 2 (telephone)					
Adherence full dose (%)					
Adherence partial dose (%)					
No. doses missed (if any)					
Week 3 (clinic)					
Adherence full dose (%)					
Adherence partial dose (%)					
No. doses missed (if any)					
Week 5 (telephone)					
Adherence full dose (%)					
Adherence partial dose (%)					
No. doses missed (if any)					
Week 7 (telephone)					
Adherence full dose (%)					
Adherence partial dose (%)					
No. doses missed (if any)					
Week 8 (clinic)					
Adherence full dose (%)					
Adherence partial dose (%)					
No. doses missed (if any)					