OxHARP

Oxford Haemodynamic Adaptation to Reduce Pulsatility Trial: Randomised, placebo controlled, double-blind crossover study of effects of sildenafil on cerebral arterial pulsatility in patients with cryptogenic or lacunar stroke and small vessel disease

Statistical analysis plan

Version 1.0

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Abbreviations

AE	Adverse event
BD	Twice a day
CO_2	Carbon dioxide
DSMB	Data and Safety Monitoring Board
MCA PI	Middle cerebral artery pulsatility index
MRI	Magnetic Resonance Image
PDE3 / PDE5	Phosphodiesterase 3/ 5
PMU	Pharmacy Manufacturing Unit
SAE	Serious Adverse Event
TCD	Transcranial Doppler
TDS	Three times a day

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1. Introduction

1.1 Purpose and scope of the plan

This document details the proposed analysis for the main paper(s) and final study report from the Wellcome Trust-funded *Oxford Haemodynamic Adaptation to Reduce Pulsatility Trial: Randomised, placebo controlled, double-blind crossover study of effects of sildenafil on cerebral arterial pulsatility in patients with cryptogenic or lacunar stroke and small vessel disease.* The results reported in these papers should follow the strategy set out here which adheres to the guidelines for the content of a statistical analysis plan[1]. Any subsequent analyses of a more exploratory nature will not be bound by this strategy though they are expected to follow the broad principles described in this document.

Suggestions for subsequent analyses by journal editors or referees will be considered carefully and carried out as far as possible in line with the principles of this analysis plan. Any deviations from the statistical analysis plan will be described and justified in the final report of the trial.

This statistical analysis plan is based on the latest version of the protocol.

1.2 Overview of the trial

Chronic injury to the small vessels of the brain ('small vessel disease') is associated with acute stroke, progressive cognitive decline, late-onset refractory depression, functional impairment in daily living and increased mortality. This study will investigate whether vasodilating medications are good candidates to reduce the progression of small vessel disease. The physiological effect of sildenafil, a phosphodiesterase 5 (PDE5) inhibitor, on cerebral arterial pulsatility will be tested, with secondary assessment of effects on cerebrovascular endothelial function. Participants will be recovered patients with a previous cryptogenic or lacunar stroke or probable TIA requiring treatment, and mild-moderate small vessel disease. Taking this population will allow assessment of effects on cerebrovascular physiology in the presence of small vessel injury, but prior to establishment of likely irreversible changes. Participants will also receive cilostazol (a PDE3 inhibitor), an active control, to compare the relative effect of PDE5 inhibition and PDE3 inhibition on cerebrovascular haemodynamics.

1.3 Trial objectives

Primary objective

To assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) reduces cerebral arterial pulsatility on transcranial ultrasound, compared to placebo in patients with chronic vascular injury to the brain (small vessel disease).

Secondary objectives

1. To assess whether 3 weeks of treatment with sildenafil alters cerebral arterial reactivity to carbon dioxide on transcranial ultrasound, compared to placebo (The null hypothesis is that there is no difference in the mean change between the sildenafil and placebo groups)
2. To assess whether 3 weeks of treatment with sildenafil has non-inferior effects on cerebral arterial pulsatility and reactivity to carbon dioxide on transcranial ultrasound, compared to cilostazol (The null hypothesis is that sildenafil is unacceptably inferior to cilostazol i.e. the change in mean flow velocity upon CO_2 challenge with sildenafil differs from that with cilostazol by more than the non-inferiority margin Δ).

3. To assess whether 3 weeks of treatment with sildenafil or cilostazol increases cerebral arterial reactivity to carbon dioxide on MRI, compared to placebo, in normal appearing white matter and within white matter hyperintensities.

Tertiary objectives (exploratory and mechanistic)

1. To assess whether 3 weeks of treatment with sildenafil or cilostazol increases cerebral arterial reactivity to carbon dioxide on MRI, compared to placebo, for all defined cerebral tissue type (all white matter and grey matter) and on a voxel-wise basis.
2. To assess whether there is an increased incidence of adverse events or serious adverse events with sildenafil or cilostazol compared to placebo.
3. To assess whether 3 weeks of treatment with sildenafil or cilostazol affects the following physiological parameters, compared to placebo:
   a) Systolic and diastolic blood pressure on 1 week of home blood pressure monitoring
   b) Arterial stiffness (carotid-femoral pulse wave velocity)
   c) Aortic pulse pressure
   d) Beat-to-beat and day-to-day variability in systolic blood pressure
   e) Flow mediated slowing
   f) Resting state cerebral autoregulation (transfer function analysis derived gain, phase and coherence between beat-to-beat blood pressure and cerebral blood flow velocity during a minimum of 5 minutes of monitoring).
   g) Alternative estimates of CVR (change in mean flow velocity per unit change in etCO2) determined during hyperventilation, 4% CO2 and 6% CO2, each versus air.

2. Trial design

OxHARP is a randomised, placebo and actively controlled, three-arm double-blind (patient and clinician), assessor-blinded crossover trial. Using random allocation, participants will have three weeks of treatment with each of the following: sildenafil (25mg TDS, titrated to 50mg TDS after one week), placebo (one tablet TDS, titrated to 2 tablets after one week), cilostazol (50mg BD, titrated to 100mg BD after one week, with midday placebo). Following each treatment period, participants will undergo a clinical and physiological assessment, followed by at least one week washout before starting the next treatment. A subgroup of participants will undergo MRI imaging following sildenafil and placebo treatments to assess arterial pulsatility on MRI and cerebrovascular reactivity, and a further subgroup will undergo MRI on all three treatments. The primary objective will be assessed using a superiority hypothesis testing framework and a non-inferiority framework will be used for the secondary objective.

2.1 Outcome measures

A detailed description of the physiological assessment measures is provided in the protocol (sections 8.5 & 8.6)

2.1.1 Primary outcome measure

Change in Gosling's middle cerebral arterial pulsatility index (MCA-PI) from baseline to end of treatment (after three weeks), utilising the visually-assessed, better quality transcranial Doppler TCD recording with a handheld probe, defined by blinded assessor.

MCA-PI will be calculated according to the formula below for each middle cerebral artery from the peak systolic velocity and end-diastolic velocity determined by operator. PSV and EDV will be determined from manually determined values across the average of three beats, during 2 recordings.

MCA-PI will be calculated for each recording, and an average of two recordings used to determine the MCA-PI for that cerebral hemisphere. 2 blinded reviewers will independently review the recordings to identify recordings that should be re-analysed or excluded due to poor quality resulting in an inaccurate value of MCA-PI. Each reviewer will also identify which side provides a better quality recording. Where no difference in recording quality between sides is evident either from visual blinded assessment or by an inconsistency in PSV of >10 cm/s, an average of the two sides will be used for the primary value of MCA-PI, otherwise the side with the better blinded quality assessment or the side with the high PSV will be used. If the PSV measured during the first 15s of the 5 minutes of TCD monitoring exceeds the PSV estimated with the hand-held probe by >10 cm/s, this period will be used to estimate MCA-PI instead.

Gosling's MCA-PI = (peak systolic velocity – end diastolic velocity)/(mean flow velocity), where flow velocity is measured in cm/s.

2.1.2 Secondary outcome measures

Percentage change in middle cerebral artery mean flow velocity per mmHg increase in concurrent end-tidal CO2 on TCD ultrasound will be estimated by the unstandardized beta-coefficient from an unadjusted linear regression between the end-tidal CO2 and MCA mean velocity during inhalation of 90 seconds of air, 2 minutes 4% CO2, 2 minutes of air and 2 minutes of 6% CO2, assessed following three weeks of treatment ('CVR'). Prior to linear regression procedure, any time delay between the etCO2 waveform and the MCA MV will be estimated by the maximum R value in a standard cross-correlation analysis, and the etCO2 trace shifted by this value. The side of insonation will be selected by the same quality assurance method as for MCA-PI. A sensitivity analysis will be performed using the standardised beta-coefficients for increase in blood flow velocity per increase in end-tidal CO2.

Cerebrovascular reactivity on MRI during inhalation of air or 6% CO2 in 2 minutes intervals (boxcar design) will be estimated as the percentage change in BOLD signal per mmHg change in end-tidal CO2, estimated from the beta-coefficient of a general linear model between each voxel time-series and a phase-shifted etCO2 trace shifted by the time-delay with the maximum r^2 by cross-correlation (FSL FEAT). The core ROIs will be normal-appearing white matter voxels and white matter voxels within a white matter hyperintensity on a 'BIANCA' mask. Secondary ROIs include total white matter, white matter hyperintensities and superficial and deep grey matter masks. BOLD scans will be preprocessed with the fMRI Expert Analsysis Tool from the fMRIB software library (FEAT), including field-map based bias field correction, high pass temporal filtering (300s cycle length), motion correction (MCFLIRT) and registration to structural (FLIRT) and standard space (FNIRT).

2.1.3 Tertiary outcome measures

a) Systolic and diastolic blood pressure will be measured as the mean of the second two readings at each sitting from day 2 to the last day of measurement
b) Beat-to-beat variability in systolic blood pressure will be measured over 5 minutes as the coefficient of variation (standard deviation / mean) after automated and manual removal of artefacts and after removal of any linear trend in the data.
c) Flow mediated slowing will be measured as the absolute reduction in speed of flow from the average value during baseline to the minimum valid reading during the first 30 seconds of recovery (assessed blind).
d) Indices of resting state cerebral autoregulation (transfer function analysis derived gain, phase and coherence) will be determined by transfer function analysis according to the

protocol recommended in the CARNET White Paper, using the open access tfa script developed by Prof Stephen Payne and made available via the CARNET collaboration.

e) A voxel-wise general linear model based analysis of cerebrovascular reactivity on MRI will be performed with the FEAT.

f) Estimates of CVR will be determined from the peak velocity achieved during each test (hyperventilation, 4% CO2 and 6% CO2) minus the baseline value, divided by the peak minus baseline etCO2 values. Results will be expressed both as absolute and percentage change in mean velocity per mmHg change in etCO2.

2.2 Randomisation and blinding

The random allocation of each participant to one of six of treatment sequences (arms) is by simple randomisation. The schedule will be created and details maintained by the Huddersfield PMU and participants will be assigned at their baseline visit. The participants, the study team and the trial statistician are blinded to treatment allocation. In participants in the MRI substudy with imaging performed only on placebo and sildenafil, the study team are unblinded to the cilostazol treatment arm, but the participants remain blinded.

2.3 Sample size

At a power level of 0.9, with a 2-sided significance of 5%, a clinically relevant 0.12 unit change in pulsatility index (equivalent to a ~20% difference in risk of recurrent stroke), and conservatively allowing for a standard deviation of differences in PI between repeated measures of 0.2, gives an estimated minimum sample size of 32 patients (paired t-test). Allowing for a 15% drop-out rate, 38 patients would be required. A sample size of 66 achieves 90% power to detect the non-inferiority of sildenafil c.f. cilostazol using a non-inferiority margin of 0.08 (and mean of paired differences 0) at α=0.025 (for a 95% CI) with a within-subject variance of 0.02. This equates to 75 patients in total with a 12% drop out rate.

2.4 Statistical interim analyses, data review and stopping guidelines

The DSMB will review recruitment and safety data after 30 patients have been recruited to the study or 12 months after the first visit of the first patient (whichever is sooner) or at the request of the sponsor or CI. Data will be reviewed in a blinded form, except where significant SAEs (in the opinion of the DSMB) or a potentially important number of severe AEs have occurred, when the data will be unblinded upon the request of the DSMB. No efficacy or futility interim analysis is planned.

If after 12 months recruitment falls below the rate expected to complete the study in 3 years, allowing for delays due to the COVID-19 outbreak, the overall study size can be reduced to 50 participants. Any data collected to that date, including on cilostazol, will be retained and analysed according to the original study plan.

The proportion of total within-patient SAEs of any grade occurring until the end of the relevant wash out period or 30 days after taking the last tablet (whichever is greater) will be compared between a) sildenafil vs placebo and b) cilostazol vs placebo using McNemar's test. "Statistically significant excess" is defined as a statistically significant result at the 5% level i.e. a test with a p-value<0.05. Any statistically significant excess of SAEs in the treatment arm, or occurrence of unexpected treatment-related SAEs of sufficient severity (in the view of the DSMB), may result in early cessation of the study. The DSMB will review the total number of SAEs and AEs on a yearly basis, and the Chair of the DSMB will be informed of any SAEs that occur within 15 days of the event.

2.5 Timing of final analysis

The primary and secondary outcomes will be analysed at the same time point, following the last visit of the last participant. Analyses corresponding to tertiary objectives will be analysed in the two years after the last visit of the last participant.

2.6 Timing of outcome assessments

The outcomes will be assessed after completion of each treatment regimen (approximately day 28, day 56, day 84). Participants who are unable to attend an on-treatment study visit once their medication has been started (for example due to the COVID-19 pandemic) will be able to restart treatment at the point of stopping treatment if still during the titration phase but otherwise will restart the three weeks of treatment. The washout period can be prolonged as long as is necessary during the COVID-19 outbreak before the next treatment phase is started. If patients experience adverse effects of treatment requiring early cessation of treatment, an early physiological assessment will be offered if possible. All outcomes will be included in the intention to treat analyses, and in per protocol analyses if more than 1 week of treatment has been given, including a dose in the last 24 hours.

2.7 Blinded analysis

A blinded analysis will be undertaken prior to the final data lock to look into the distribution of variables, missing data distributions, outliers and to finalise the per protocol population.

3. Statistical principles

3.1 Statistical significance and multiple testing

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. 95% confidence intervals presented will be at 95% and two-sided. The 97.5% upper limit will be used in the non-inferiority analysis. No adjustment for multiplicity is planned.

3.2 Adherence and protocol deviations

Compliance with study drugs is assessed by direct questioning at each follow-up visit and by the counting of returned study drug. The number and percentage of participants who adhered to each treatment regimen will be presented in a table by treatment period and group, and overall. Adherence to treatment is defined as taking at least 80% of prescribed medication during the treatment period (including if treatment ceases early but a physiological assessment is performed), including 3 doses in the last 26 hours before outcome assessment. Protocol deviations (such as errors in applying inclusion/exclusion criteria and missed follow-up visits) will be defined as major or minor prior to unblinding of data, defined in the protocol violation log stored on the CPSD High Compliance Server. The number of patients with major protocol deviations, and the type of deviation will be summarised by treatment sequence group. No formal statistical testing will be undertaken.

3.3 Analysis populations

Intention to treat population The intention to treat (ITT), or full analysis set, population will be all participants randomised, irrespective of eligibility or treatment received. This ITT population will be used for the final analysis corresponding to the primary aim for all participants with available primary outcome data included, and including all patients receiving at least 1 tablet of medication for safety outcomes.

Per protocol population All participants in the ITT population who

- complete each of the treatments for each analysis and

- have no major protocol deviations, prior to unblinding or analysis, leading to exclusion
- are not excluded due to other reasons defined prior to analysis/unblinding
- have had a physiological assessment following at least 1 week of continuous treatment and three trial doses in the last 26 hours.

Safety population

The safety population will consist of all participants who have received at least part of one dose of treatment. This population will be used to summarise the safety (adverse event) data.

4. Descriptive analyses

4.1 Participant throughput

A flow chart template based on the CONSORT guidelines for crossover trials[4] is provided in Appendix 1, indicating the progress of participants through each stage of the trial.

4.2 Withdrawal from treatment and/or follow-up

Withdrawals from treatment will be reported by treatment group and by arm (treatment sequence allocation group) and specific timepoint. Study withdrawals and loss-to-follow up will also be reported according to treatment sequence allocation and timing of withdrawal (see flow chart template). The proportion of missing outcome data will be given.

4.3 Baseline characteristics

The baseline characteristics of participants will be described, both overall and by treatment sequence allocation group. This will include at least age, gender, incident event (TIA vs stroke), cardiovascular risk factors (history of hypertension, diabetes, smoking), use of concurrent antihypertensive medication and antiplatelet treatment and severity of white matter hyperintensities on eligibility scan (Fazekas scale for MRI, modified Blennow scale for CT). For participants in the MRI substudy, volume of white matter hyperintensities will also be reported. Tests of statistical significance will not be undertaken.

4.4 Unblinding

Any cases of treatment unblinding not pre-specified in the protocol will be listed, together with the reasons for unblinding.

5. Comparative analyses

5.1 Primary outcome

Gosling's MCA-PI will be compared between sildenafil and placebo treatment by paired t-test and a 95% confidence interval if normality distribution assumptions hold, or the non-parametric Wilcoxon signed rank test. If the placebo phase is not available, a comparison between the baseline value and sildenafil period will be accepted. The analysis will be re-run using the PP population as a sensitivity analysis, and using the percentage change at each phase from baseline.

5.2 Secondary outcomes

The percentage change in mean blood flow velocity per mmHg during inhalation of air, 4% and 6% carbon dioxide versus medical air estimated from the beta-coefficient from a phase-shifted linear regression will be compared between sildenafil and placebo treatment by paired t-test and a 95% confidence interval if normality distribution assumptions hold, or the non-parametric Wilcoxon signed rank test. The analysis will be re-run using the PP population as a sensitivity analysis, and using the percentage change from baseline.

A non-inferiority analysis will be based on the 95% CI for the mean change difference between sildenafil and cilostazol. If the upper 97.5% confidence limit is less than 0.08 units (=inferiority margin, 2/3 of the clinically estimated overall cilostazol effect), non-inferiority will be declared. The data will be transformed if normality assumptions do not hold. The PP population will be used for this analysis, with the ITT population being used in a sensitivity analysis.

Differences in reactivity to CO_2 from baseline in each of three states (sildenafil, cilostazol, placebo) will be compared by mixed-effect general linear models, with post-hoc pairwise comparison, using the ITT population with the PP population being used in a sensitivity analysis. This will apply to both TCD measures of CO2 reactivity and by ROI on MRI. On MRI, these analyses will also be performed on a voxel-wise basis by use of the FLAME tool (FSL).

5.3 Missing data

All physiological data will reviewed and cleaned by automated and manual processes to reduce artefactual data. The completeness and correctness of the data will be monitored as per the monitoring plan. Derived indices will be calculated from raw data by assessors blind to treatment allocation, and for primary and secondary analyses will be performed before data lock. No missing data adjustments will be used, except where a valid baseline measure is missing. In these circumstances, a baseline value will be imputed from the whole population data, using placebo arms only. Analyses will be run with and without outliers (spurious data).

5.4 Protocol pre-specified tertiary/exploratory analyses

The imaging analysis is specified in the protocol and this document. This will be completed and followed by the CI and relevant team members. Blood will be stored and then analysed at completion of the study, as the extent of testing will depend upon the demonstrated effect of medications in the study, whilst allowing for assessment of new biochemical and genetic markers reported during the study. Analysis of the markers of endothelial function and genetic targets of the trial medications (to be analysed in the two years after the last visit of the last participant) will also be detailed separately.

6. Safety data

Safety data will be routinely collected at each follow-up, including clinical history of side effects or adverse events, clinical examination and blood tests (FBC, U+Es, LFTs). The safety population will be used to summarise the adverse event data.

Adverse events (AEs) will be presented in tabular form a) per participant (see e.g. Table 6.1 below; all patients taking at least one tablet will be included) and b) per treatment administered in each period (e.g. see Table 6.2 below) including the wash-out (to a maximum of 30 days since the last tablet was taken). For each treatment within each patient, only the maximum severity of each type of AE will be displayed.

Table 6.1 Example of AE table

Adverse event (AE)		No. of participants	No. of AEs
e.g. Headache	No AE under any of the three treatments		
	No AE under sildenafil or cilostazol but AEs observed under placebo		
	No AE under sildenafil but AEs observed under placebo and cilostazol		

	AE observed under sildenafil but not under cilostazol or placebo		
	AE observed under sildenafil and cilostazol but not placebo		
	AE observed under sildenafil, cilostazol and placebo		

Table 6.2 Example of AE table

Period	Treatment	No. of participants taking treatment	No. of mild AEs	No. of moderate AEs	No. of severe AEs
First	Sildenafil				
	Cilostazol				
	Placebo				
Second	Sildenafil				
	Cilostazol				
	Placebo				
Third	Sildenafil				
	Cilostazol				
	Placebo				

SAEs are reportable for up to 30 days following the last administration of a trial drug whether after the end of a trial or between trial phases following a prolonged washout period and will be listed in full.

7. Statistical software

All analysis will be carried out using appropriate validated statistical software such as STATA, SAS or R. The relevant package and version number will be recorded in the final report.

8. References

1. Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, Williamson PR, Altman DG, Montgomery A, Lim P, Berlin J, Senn S, Day S, Barbachano Y, Loder E. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA 2017;318(23):2337-2343

2. Julious SA, Campbell MJ, Altman DG. Estimating sample sizes for continuous, binary, and ordinal outcomes in paired comparisons: practical hints. J Biopharm Stat. 1999;9(2):241-251.

3. Julious SA. Sample sizes for clinical trials with normal data. Stat Med. 2004;23(12):1921-1986

4. CONSORT 2010 statement: extension to randomised crossover trials BMJ 2019;366:l4378 https://www.bmj.com/content/366/bmj.l4378

https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-9-statistical-principles-clinical-trials-step-5_en.pdf

9. Document history

Version	Date	Comments/justification	Protocol version	Timing in relation to unblinding of trial statistician
0.1	07Mar2021	First draft	3.0	Prior to unblinding
0.2	19Jan2022	Second Draft	3.0	Prior to unblinding
0.3	25/06/2022	First approved version	3.0	Prior to unblinding
0.4	31/01/2023	Revision of Approved version	3.0	Prior to unblinding
0.5	27/02/2023	Revision of Approved version	3.0	Prior to unblinding

Appendix 1. Flow chart template - stored separately.