The PASTIS trial. Testing tadalafil for possible use in vascular cognitive impairment

Mathilde MH Pauls et al.

Research in Context

Systematic review: Brain vascular disease is a major contributor to dementia, with few treatment options. Phosphodiesterase inhibitors (PDE5i) are widely-used vasodilators in erectile dysfunction. Hence, we tested whether tadalafil, a brain-penetrant PDE5i, increases deep brain blood flow.

Interpretation: This is the first randomised clinical trial of a PDE5i in small vessel disease. The data did not support a difference between single-administration tadalafil and placebo with respect to subcortical blood flow. In order to increase CBF, combination therapy may be required to over-ride CBF autoregulation. A trend to augmented perfusion within white matter hyperintensities (9.8%, P=0.096) suggested that PDE5i treatment for longer duration may have clinical benefit.

Future directions: A future trial will require a longer treatment regimen in older people (age ≥ 65) with sufficient power to detect 10% blood flow augmentation in white matter hyperintensities. [136 words < 150]

Abbreviations: PDE5i: phosphodiesterase inhibitor

The PASTIS trial. Testing tadalafil for possible use in vascular cognitive impairment

Highlights

Single administration of the PDE5 inhibitor tadalafil

- was well-tolerated in older people with symptomatic small vessel disease.
- reduced SBP and DBP.
- did not reduce cerebral blood flow (measured with ASL).
- trended enhanced perfusion in white matter hyperintensities.

The PASTIS trial. Testing tadalafil for possible use in vascular cognitive impairment [85 < 100 characters]

Mathilde MH Pauls[1,2], Lauren R Binnie[1], Philip Benjamin[1,3], Shai Betteridge[4], Brian Clarke[2], Mohani-Preet K Dhillon[1], Rita Ghatala[5], Fearghal AH Hainsworth[1], Franklyn A Howe[1], Usman Khan[2], Christina Kruuse[6], Jeremy B Madigan[3], Barry Moynihan[2,7], Bhavini Patel[2], Anthony C Pereira[1,2], Egill Rostrup[8], Anan BY Shtaya[1], Catherine A Spilling[1], Sarah Trippier[5], Rebecca Williams[5], Robin Young[9], Thomas R Barrick[1], Jeremy D Isaacs[1,2] and Atticus H Hainsworth[1,2] *

[1]Molecular & Clinical Sciences Research Institute, St George's University of London, UK; [2]Department of Neurology, St George's University Hospitals NHS Foundation Trust, London, UK; [3]Neuroradiology, St George's University Hospitals NHS Foundation Trust, London, UK; [4]Neuropsychology, St George's University Hospitals NHS Foundation Trust, London, UK; [5]South London Stroke Research Network, London, UK; [6]Department of Neurology and Neurovascular Research Unit, Herlev Gentofte Hospital, Denmark; [7]Department of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland; [8]Mental Health Centre, University of Copenhagen, Glostrup, Denmark; [9]Robertson Centre for Biostatistics, University of Glasgow, UK.

*correspondence: Dr AH Hainsworth PhD, St George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom. Tel:+442087255586 fax:+442087252950. email: ahainsworth@sgul.ac.uk

Running title: Tadalafil and CBF in small vessel disease

Tables: 2; Figures: 4, black & white

Word count: 3362 <3,500 words (excluding the abstract, references, figures, and tables)

Abstract

INTRODUCTION: There are few randomized clinical trials in vascular cognitive impairment. This trial tested the hypothesis that the PDE5 inhibitor tadalafil, a widely-used vasodilator, increases cerebral blood flow (CBF) in older people with symptomatic small vessel disease, the main cause of VCI.

METHODS: In a double-blinded, placebo-controlled, cross-over trial, participants received tadalafil (20mg) and placebo on two visits ≥7 days apart (randomized to order of treatment). The primary endpoint, change in subcortical CBF, was measured by arterial spin labelling.

RESULTS: Tadalafil increased CBF non-significantly in all subcortical areas (N=55, age: 66.8 (8.6) years) with greatest treatment effect within white matter hyperintensities (+9.8%, P=0.0960). There were incidental treatment effects on systolic and diastolic blood pressure (-7.8, -4.9 mmHg; P<0.001). No serious adverse events were observed.

DISCUSSION: This trial did not identify a significant treatment effect of single-administration tadalafil on subcortical CBF. To detect treatment effects may require different dosing regimens. [149 words < 150]

Abbreviations: CBF: cerebral blood flow

Clinical Trial Registration. http://www.clinicaltrials.gov. Unique identifier: NCT00123456. https://eudract.ema.europa.eu. Unique identifier: 2015-001235-20NCT00123456.

Keywords: clinical trials; Tadalafil; PDE5; cerebral blood flow; small vessel disease; vascular cognitive impairment; VCID

Abbreviations and Acronyms (Word Count: 52)

CBF: cerebral blood flow

DGM: deep grey matter

MoCA: Montreal Cognitive Assessment

NAWM: normal appearing white matter

NIHSS: National Institutes of Health Stroke Scale

PASTIS: Perfusion by Arterial spin labelling following Single dose Tadalafil In Small vessel disease

PDE5i: phosphodiesterase-5 inhibitors

SVD: small vessel disease

WMH: white matter hyperintensities

VCI: vascular cognitive impairment

1. Background

Small vessel disease (SVD) is a common cause of lacunar stroke and vascular contributions to cognitive impairment and dementia [1, 2]. SVD is common in older people, seen on brain MRI as diffuse white matter hyperintensities (WMH), focal ischemic lesions and micro-haemorrhages [2]. SVD is associated with reduced cerebral blood flow (CBF) particularly in subcortical areas, including deep grey nuclei, subcortical white matter and within WMH [3-7]. There is currently no disease-modifying therapy for SVD [2, 8].

CBF is regulated by multiple factors, including nitric oxide (NO). Tonic endothelial NO activates guanylyl cyclase in overlying vascular myocytes, to drive cGMP formation, leading to myocyte relaxation and vasodilation. Cytoplasmic cGMP is degraded by phosphodiesterase enzymes, in particular PDE5. Potent, selective PDE5 inhibitors (PDE5i) such as sildenafil (Viagra®) and tadalafil (Cialis®) are in routine use as vasodilators in erectile dysfunction and pulmonary arterial hypertension. PDE5i augment blood flow in peripheral tissues and are well-tolerated across dosing regimens [9, 10]. This study addressed the hypothesis that PDE5i increase CBF in older people, particularly in the subcortical regions affected by SVD [11].

PDE5 is present in human brain neurons [12] and in vascular myocytes within subcortical white matter [13]. Among PDE5i, tadalafil has a relatively-long plasma half-life (16 h in healthy adults) [14, 15] with evidence of brain penetration in rodents and primates [16, 17]. Tadalafil is well-tolerated and has been widely-prescribed worldwide [9, 10, 14, 15]. This paper presents primary outcomes from a clinical trial with crossover design [8, 18] to determine whether a single administration of tadalafil increases subcortical CBF.

2. Methods

For Expanded Methods please see the Supplemental file.

This trial was preregistered at http://www.clinicaltrials.gov (Unique identifier: NCT00123456) and https://eudract.ema.europa.eu (Unique identifier: 2015-001235-20NCT00123456).

The data supporting this report are available from the corresponding author upon reasonable request.

2.1 Trial Design, Randomisation and Endpoints.

The trial received ethical approval from the UK National Research Ethics Service (REC reference: 15/LO/0714). Within the UK the National Research Ethics Service, part of the NHS Health Research Authority (https://www.hra.nhs.uk/) enacts the principles of the Declaration of Helsinki (and subsequent amendments; World Medical Association) for medical research involving human subjects. Written informed consent was obtained from all participants or their next of kin. Participants were enrolled by members of the trial team and randomised to order of treatment (tadalafil 20 mg, placebo; oral administration). The randomisation list was generated in advance by Sharp Clinical Services, Crickhowell, Powys, UK. Each participant received on two separate occasions, visit#1 and visit#2, a placebo dose and a tadalafil 20mg dose which were identical in size, shape, weight and colour. Two study visits were performed at least 7 days apart, with blood pressure measurement, MRI scanning and a battery of cognitive tests up to 3 h before and 3-5 h after dosing (see Fig.1A). Participants, care providers and those assessing outcomes were all blind to treatment allocation.

The primary endpoint was change in subcortical CBF, assessed in three tissue types: deep grey matter nuclei (DGM), normal appearing white matter (NAWM) and WMH. Change in CBF for cortical grey matter was a secondary endpoint.

The trial commenced 4th September 2015 (Fig.1B). Participants were recruited from St George's Hospital and local Participant Identification Centres. All visits, data management and trial coordination were performed at the St George's site. The trial ended when the pre-determined recruitment target was met (25 January 2018).

2.2 Study Population.

All data were from older adults without known diagnosis of dementia, with radiological and clinical evidence of symptomatic SVD. Inclusion criteria were as follows. 1, radiological evidence of SVD, defined as: MRI evidence of lacunar infarct(s) up to 15 mm maximum diameter and/or confluent deep WMH (grade 2 or higher on the Fazekas scale [19]). 2, clinical evidence of SVD defined as either: lacunar stroke syndrome with symptoms lasting at least 24 hours, occurring at least 6 months prior to visit#1; or: transient ischaemic attack lasting <24 hours with limb weakness, hemi-sensory loss or dysarthria at least 6 months previously and with diffusion-weighted MRI performed acutely showing lacunar infarction. If MRI was not performed within 10 days of transient ischaemic attack, a lacunar infarction in an anatomically appropriate position as demonstrated on a subsequent MRI was also deemed eligible. 3, Age at least 50 years. 4, imaging of the carotid arteries in the previous 12 months, demonstrating less than 70% stenosis in both internal carotid arteries or less than 50% stenosis in both internal carotids if measured in previous 12-60 months. Exclusion criteria included: known diagnosis of dementia; cortical infarction (more than 15 mm maximum width); SBP<90 mmHg; DBP<50 mmHg; creatinine clearance <30ml/min; stroke

or TIA within the previous 6 months; concomitant use of PDE5i. A full list of exclusion criteria is given in the published protocol [18].

2.3 Study Assessments.

In the screening visit ("Visit 0") informed consent was documented and education level and Montreal Cognitive Assessment (MoCA) scores were recorded. In study visits (Visit#1, Visit#2) participants underwent blood pressure measurements, a cognitive test battery [18] and brain MRI. At the end of each study visit, and at least 3 h post dosing, two blood samples (5 ml) were taken for full blood count and analysis of tadalafil concentration.

2.4 MRI Acquisition.

Whole-brain perfusion MRI was acquired using a 3T scanner (Achieva Dual TX MRI scanner, Philips Medical Systems, Eindhoven, Netherlands) at St George's University Hospitals NHS Foundation Trust. Whole brain T1-weighted, Fluid Attenuated Inversion Recovery (FLAIR), susceptibility-weighted imaging (SWI) and pseudo-continuous arterial spin labelling (pCASL) images (which included a proton-density weighted image) were acquired. Full MRI acquisition protocol information is provided in the online supplement. All MRI data were acquired from brain scans performed on a Tuesday or Thursday, pre-dosing scans between the hours of 10:00 and 12:00 and post-dosing scans 14.00-17.00. The pCASL protocol was based on the consensus recommendations of the ISMRM Perfusion study group and European consortium for ASL in dementia [20] using the Philips pCASL sequence in the scanner v5.3 software.

2.5 MRI Analysis.

Full MRI analysis information is included in the online supplement and Supplemental Fig. S1 provides an outline of the MRI data analysis pipeline. An average pCASL map was separately computed for each pCASL data acquisition (example in Fig.2) using *oxford_asl* (part of the FSL-BASIL toolset, fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL). CBF in each voxel was calculated using the standard equation for pCASL [20].

For each scanning session, T1-weighted images in native space were segmented into grey matter, white matter and CSF tissue probability maps (Fig.2) using a modified form of the standard Statistical Parametric Mapping (SPM v12, https://www.fil.ion.ucl.ac.uk/spm/) described in our previous paper [21]. WMHs were delineated on FLAIR images (JIM software v7.0, Xinapse Systems Ltd, West Bergholt, Essex, UK). Native space T1-weighted and FLAIR images were co-registered to the average proton density-weighted image, to enable alignment of the T1-weighted tissue probability and WMH maps to the CBF data.

Each voxel in the CBF map was provisionally assigned to either grey matter, NAWM, WMH or CSF, based on the maximum tissue probability. These provisional assignments were then entered as empirical priors in a Hidden Markov random field model and segmentation (FMRIB's Automated Segmentation Tool, FAST) [22] to provide an improved segmentation of grey and white matter tissue from the CBF maps. This technique reduces partial volume and tissue classification errors at the boundary between grey and white matter, caused by the relative difference between voxel sizes of the native pCASL and T1-weighted images. For each participant at each scan session, median CBF values were calculated for total grey matter, NAWM and WMH (example in Fig.2).

To determine CBF in DGM, the caudate, putamen and thalamus of both hemispheres were segmented on native space T1-weighted images using Freesurfer (Ver.5.3.0,

https://surfer.nmr.mgh.harvard.edu/fswiki/). Median CBF was calculated for each of these three deep grey nuclei and the average of these median values reported as CBF in DGM.

2.6 Statistical Analysis.

All analyses were based on the intention-to-treat principle (i.e. participants were analyzed according to randomized treatment group regardless of whether they received the intended treatment). Change within each treatment group was analysed using paired sample t-tests. Treatment effects were defined as {(after-before tadalafil)-(after-before placebo)}. Treatment effects on primary and secondary outcomes were analysed using linear mixed effects models with fixed effects of baseline value, treatment, visit and random effect of subject. Models were not corrected for age, blood pressure or full blood count. Analyses was conducted using R v.3.4.1 with the lme4 and lmerTest packages (https://www.R-project.org/). No corrections were made for multiple comparisons. $p<0.05$ was considered significant.

3. Results

Sixty-five individuals gave consent and were randomized, 59 commenced the protocol, 55 completed the protocol and 53 had a full set of usable CBF data (see CONSORT diagram, Fig.1C). There were no significant demographic differences between those randomized and those who completed the protocol (Table 1).

Ten participants experienced adverse events, eight while on placebo treatment and two while on tadalafil (described in supplemental Table S1). These included headache, nausea, sore throat, knee pain, respiratory infections, a diabetic hypoglycaemic event, a panic attack in the MRI scanner. There were no serious adverse reactions. The cohort were older adults (age

range: 52-87 years, Table 1) all of whom had symptomatic SVD with typical MRI manifestations. Though the protocol permitted inclusion of TIA patients, in practice all had a lacunar stroke event, verified on MRI. In all cases, visit#1 took place at least six months post-stroke. Visit#1 and visit#2 were 20 (19) days apart (mean (SD); range 7-117 days). Four participants completed visit#2 more than 30 days after visit#1 (range 54-117 days). In blood samples taken 3-6 hours post-drug administration plasma tadalafil concentration was 520 (160) nmol/L (range 300-980 nmol/L, n=53).

Following tadalafil administration, CBF increased in all three subcortical tissue types (DGM, NAWM and WMH) as well as in total grey matter (Fig.3A-D; Table 2). CBF also increased following placebo in DGM, NAWM and total grey matter, but not in WMH (Table 2). Treatment effects of tadalafil on CBF were modest and not significant: 0.11 ml/min/100g in DGM ($p=0.881$), 0.33 ml/min/100g in NAWM ($p=0.458$), 0.95 ml/min/100g in WMH ($p=0.0960$) and 0.56 ml/min/100g in total grey matter ($p=0.456$). The highest treatment effect was in WMH, representing a 9.8% increase in CBF. There was no significant effect of group allocation (tadalafil at visit#1 and placebo at visit#2, or vice versa). No significant carry-over effect was detectable in any of the statistical models ($p>0.180$ for all treatment-period interactions).

As incidental findings, there were modest but significant treatment effects on SBP (-7.8 mmHg, $p<0.001$) and DBP (-4.9 mmHg, $p<0.001$) in post hoc analyses (Fig.3E-F; Table 2).

4. Discussion

This paper reports primary outcomes from the first double-blinded, randomized placebo-controlled clinical trial of PDE5i treatment on CBF in older people with SVD. The main finding was that single administration of tadalafil did not significantly increase CBF relative to placebo in subcortical tissue or in total grey matter (which is dominated by cortical grey matter). As an incidental finding, tadalafil decreased SBP and DBP relative to placebo.

The dose of tadalafil (20 mg) was within the range licensed for prescribing (5-40 mg) and between the dose typically prescribed in erectile dysfunction (5-10 mg) and that used in clinical trials for pulmonary arterial hypertension (40 mg). Tadalafil-dependent reduction in blood pressure confirmed active plasma concentrations of drug. The plasma concentrations recorded were consistent with previous pharmacokinetic studies [14, 15]. Based on the published brain:plasma ratio of approximately 1:10 in rodents and experimental primates [16, 17], brain tadalafil concentration in the range 30-100 nmol/L is estimated for the participants in PASTIS. Hence, brain tadalafil levels in this trial were likely to be at least 6-fold above the concentration required for half-maximal PDE5 inhibition (pharmacological IC_{50} values in the range 1-5 nmol/L).

An unexpected finding was significant CBF elevation in DGM, NAWM and total grey matter following placebo treatment. There was no effect of order of treatment (group allocation). Considering total grey matter, where the signal-noise ratio for CBF measurement is highest, the post-placebo increase was evident in 41 (72 %) participants who received placebo. This appears a high proportion for a true placebo effect. Alternatively, the observation may reflect diurnal CBF variation, possibly mediated by circadian changes in circulating hormones, vasomotor tone or psychological arousal. Cyclical variations in haemodynamic parameters, including CBF, are reported in experimental animals [23] and in human subjects [24-27].

This unexpected finding emphasizes the need for a placebo group in studies of CBF, even where a cross-over design is used. Future trials should specify the time of day for CBF measurement.

Tadalafil-mediated treatment effects on CBF were small (range 0.4-9.8% across tissue types) and non-significant (Table 2). As grey matter CBF data have high signal/noise and statistical power, the findings suggest that tadalafil-mediated grey matter CBF changes are unlikely, at least in a single-dosing regimen. Failure of target engagement is unlikely, as brain tadalafil concentration is estimated to be high and PDE5 is present in vascular myocytes of older people [13]. The greatest treatment effect was within WMH, equivalent to a 9.8% increase ($p=0.0960$, Table 2). This finding raises two questions. First, is this a true increase (and our finding a false negative)? This trial was designed to detect treatment effects of 15% or more [18]. To confidently detect a 9.8% increase in perfusion of white matter tissue would require a substantially larger cohort. Second, what would be the clinical impact of a 9.8% increase in WMH blood flow? Such an increment, if confirmed, would at least indicate that small vessels within WMH are amenable to pharmacotherapy. Previous studies support the concept that CBF regulation within WMH differs from surrounding NAWM [3, 5, 6]. Given the nonlinear relation between brain perfusion and tissue damage, it is conceivable that such a modest elevation in local blood flow could be beneficial. In other tissues, chronic PDE5i treatment produced therapeutic myocardial remodeling in heart failure [28] and in pulmonary arterial hypertension [29]. Post hoc analyses revealed a trend for greater post-tadalafil CBF change with increasing age (Fig.4). It appears reasonable to speculate that putative PDE5i-mediated effects on CBF may be most apparent in older persons, at least 65 years of age.

Changes in CBF following PDE5i administration have been reported in older people with brain disease [30-32] though the results were quite diverse. In older male subjects with a history of ischemic stroke (combining large vessel and lacunar subtypes) a mosaic of changes in regional CBF followed tadalafil treatment [31]. Sildenafil also gave a mosaic of regional CBF changes in men with erectile dysfunction [30]. In subjects with mild cognitive impairment, diagnosed clinically as "early Alzheimer's Disease", there was a small (8%) elevation of global CBF following acute administration of sildenafil (50 mg) [32]. All these studies lacked a placebo-treated control group [30-32], hence small PDE5i-dependent effects cannot be distinguished from confounding factors, such as diurnal CBF changes [27]. Healthy young adults showed no change in CBF following acute PDE5i treatment [33, 34]. In young adult male patients with Becker muscular dystrophy, four weeks of sildenafil treatment produced a small increase in cerebrovascular reactivity (1.6%) though CBF did not change significantly [35].

Other vasoactive agents that have been tested in older people, either with documented hypertension or with a history of SVD, include angiotensin converting enzyme inhibitors [36, 37], angiotensin receptor antagonists [38, 39], beta-adrenoceptor blockers [36] and calcium channel antagonists [40]. All reduced systemic blood pressure without significant effects on CBF. Most of these prior studies employed drug treatment for at least 2 weeks in small cohorts (8-28 participants). In a larger study of 67 hypertensive older people with SVD, either intensive or standard ABP lowering treatment achieved 27 mmHg. 8 mmHg fall in SBP, respectively [41]. Following 3 months of treatment there was no significant change in global CBF from baseline in either group, and no difference in CBF between the intensive and standard treatment groups [41]. There are exceptions to this pattern. A modest, significant increase in grey matter CBF (9.5%) was reported in older hypertensive patients

following intensive blood pressure lowering, relative to those on usual blood pressure treatment [42]. In Alzheimer's patients, 6 months of treatment with the calcium antagonist nilvadipine suppressed blood pressure with no change in global CBF, though post hoc analyses showed a substantial (20%) increase in hippocampal CBF [43].

The present results and the bulk of published data [36-41, 44] support the view that CBF autoregulation is maintained following drug treatment, even in older people with manifest SVD. The present findings support the safety of tadalafil in older people with SVD. Sustained CBF augmentation may require a combination of interventions [45, 46].

The interplay between hypertension, SVD and cognitive decline is an area of high interest. A recent large study (SPRINT-MIND) compared intensive with standard blood pressure lowering (target SBP<120 mmHg, <140 mmHg, respectively) [47]. While there was no difference in the primary outcome of incident dementia (median treatment duration 3.3 years) intensive treatment significantly reduced risk of mild cognitive impairment and the combined risk of MCI or probable dementia [47]. In a subgroup with MRI data, WMH volume increase was significantly lower in the intensive treatment group (between-group difference: 540 mm^3, P<0.001) [48]. These findings support an earlier study (PROGRESS) where blood pressure lowering (using an ACE inhibitor combined with a diuretic) reduced WMH accumulation and lowered the risk of cognitive decline [46]. Recent meta-analyses support a beneficial effect of midlife blood pressure lowering [49]. Overall, these findings suggest that cognitive impairment due to SVD may be tractable to intensive antihypertensive strategies.

This study has strengths. First, it recruited a well-characterized cohort with clinical and MRI evidence for symptomatic SVD. Second, CBF maps were derived from a long pCASL sequence (20 minutes), all performed on a single MRI scanner. Third, sufficient participants were recruited to attain the pre-determined statistical power.

This study also has limitations. First, only a single administration of tadalafil was examined. While this was anticipated to attain the expected biological effect of near-complete PDE5 inhibition, additional effects could emerge from a longer dosing regimen. Second, relatively-young participants were included (age ≥50 y, Table 1). Of the 55 who completed the protocol, 22 (40%) were aged <65y. As tadalafil does not affect CBF in young, healthy adults [33, 34], this may have diluted a possible treatment effect. Third, only resting CBF was measured. It may be that PDE5i affect changes in cerebrovascular reactivity in response to a stimulus (such as a visual image, breath-holding or motor task) [35].

Conclusion

This study found insufficient evidence to support a significant difference between single dose tadalafil (20 mg) and placebo with respect to increase in resting subcortical CBF. Modest reduction in blood pressure was observed and did not result in hypoperfusion in SVD. A trend to augmented WMH perfusion suggests that PDE5i treatment, possibly of longer duration, may yield clinical benefit.

Acknowledgements

We thank our colleagues at St George's, in particular the Atkinson-Morley Radiography team, Stroke service, South London CRN Stroke Research Network, Joint Research and Enterprise Office and Clinical Research Facility. We acknowledge our late colleague and friend Debbie Rolfe (deceased) for her support. AHH thanks Dr HMTC Mulrooney for helpful comments.

Disclosures/Conflict of Interest. MMHP and LRB were employed as part of the PASTIS trial, JDI was Principal Investigator and AHH was Chief Investigator. CK is a PI on clinical trials with Bristol-Myers-Squibb and Bayer, and has received funding from NovoNordisk, Bayer and Bristol-Myers-Squibb, all not relevant to the present trial. JDI has been a PI on clinical trials funded by Roche, Merck and Lupin Pharmaceuticals and has received funds from Biogen and Roche, none relevant to the present trial. AHH has received honoraria from Eli-Lilly and from NIA, he chairs the Vascular Cognitive Disorders PIA within ISTAART and he leads MRC-Dementias Platform UK Vascular Experimental Medicine group. All other authors report no relevant disclosures. The trial was subject to an ICH-Good Clinical Practice (GCP) inspection by the UK medicines regulator, the MHRA, in September 2019 which identified a number of regulatory findings associated with the management of the trial. These are outlined in the supplementary information.

Sources of Funding. This study was joint-funded by UK Alzheimer's Society and Alzheimer's Drug Discovery Foundation (Grant Ref. 20140901).

The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for

publication. The sponsor was St George's University of London (contact: sponsor@sgul.ac.uk).

Supplemental Materials.

Expanded Methods.

Supplementary Figure S1, MRI data analysis workflow.

Supplemental Table S1, list of adverse events.

Figure Legends

Figure 1. PASTIS trial design and recruitment. A, trial design. For Group 1, Treatment 1 was tadalafil and Treatment 2 placebo, for Group 2 vice versa. B, Recruitment to PASTIS. C, CONSORT diagram.

Figure 2. Example of anatomical and CBF mapping, with tissue segmentation. A, FLAIR image at full resolution. B, FLAIR image co-registered to the CBF map, with voxels re-sized to be equivalent to the pCASL map. C, CBF map, derived from pCASL. Calibration bar shows 0.0-80.0 ml/min/100g. D, tissue segmentation map for CBF computation. Each voxel has been defined as either: cerebrospinal fluid (CSF), grey matter (GM), normal-appearing white matter (WM) or white matter hyperintensity (WMH). E, F: probability density functions of CBF values in voxels assigned as grey matter (E) or normal-appearing white matter (F). For this participant, median CBF was 51.3 ml/min/100g in grey matter and 21.8 ml/min/100g in normal-appearing white matter.

Figure 3. Distributions of change (after – before) following placebo or tadalafil for CBF and blood pressure. A-D: change in CBF (ml/min/100g) in Deep Grey Nuclei (A), normal-appearing white matter (B), White Matter Hyperintensities (C) and total grey matter (D). E-F: change in SBP (E) and DBP (F) (mmHg). Box-whisker plots show median, IQR and full range. Asterisks indicate mean values. For each of the parameters presented, mean values are listed in Table 2. Individual data points shown are >3 IQR from the median.

Figure 4. Change in CBF following placebo or tadalafil as a function of age. Change in CBF in total grey matter (A), and normal-appearing white matter (B). Lines of best fit are shown for placebo (solid line), or tadalafil (dashed line) or for all data points (grey line).

References

[1] M.M. Esiri, G.K. Wilcock, J.H. Morris. Neuropathological assessment of the lesions of significance in vascular dementia. J Neurol Neurosurg Psychiatry. 1997;63:749-753.

[2] R.J. Cannistraro, M. Badi, B.H. Eidelman, D.W. Dickson, E.H. Middlebrooks, J.F. Meschia. CNS small vessel disease: A clinical review. Neurology. 2019;92:1146-1156.

[3] M. O'Sullivan, D.J. Lythgoe, A.C. Pereira, P.E. Summers, J.M. Jarosz, S.C. Williams, H.S. Markus. Patterns of cerebral blood flow reduction in patients with ischemic leukoaraiosis. Neurology. 2002;59:321-326.

[4] H. Yao, S. Sadoshima, S. Ibayashi, Y. Kuwabara, Y. Ichiya, M. Fujishima. Leukoaraiosis and dementia in hypertensive patients. Stroke. 1992;23:1673-1677.

[5] M. Bernbaum, B.K. Menon, G. Fick, E.E. Smith, M. Goyal, R. Frayne, S.B. Coutts. Reduced blood flow in normal white matter predicts development of leukoaraiosis. J Cereb Blood Flow Metab. 2015;35:1610-1615.

[6] J.W. van Dalen, E.P. Moll van Charante, M.W.A. Caan, P. Scheltens, C.B.L.M. Majoie, A.J. Nederveen, W.A. van Gool, E. Richard. Effect of Long-Term Vascular Care on Progression of Cerebrovascular Lesions: Magnetic Resonance Imaging Substudy of the PreDIVA Trial (Prevention of Dementia by Intensive Vascular Care). Stroke. 2017;48:1842-1848.

[7] P.H. van der Veen, M. Muller, K.L. Vincken, J. Hendrikse, W.P. Mali, Y. van der Graaf, M.I. Geerlings, S.S. Group. Longitudinal relationship between cerebral small-vessel disease and cerebral blood flow: the second manifestations of arterial disease-magnetic resonance study. Stroke. 2015;46:1233-1238.

[8] E.E. Smith, H.S. Markus. New Treatment Approaches to Modify the Course of Cerebral Small Vessel Diseases. Stroke. 2019:STROKEAHA119024150.

[9] R.A. Kloner, G. Jackson, A.M. Hutter, M.A. Mittleman, M. Chan, M.R. Warner, T.M. Costigan, G.M. Vail. Cardiovascular safety update of Tadalafil: retrospective analysis of data from placebo-controlled and open-label clinical trials of Tadalafil with as needed, three times-per-week or once-a-day dosing. Am J Cardiol. 2006;97:1778-1784.

[10] R.A. Kloner, I. Goldstein, M.G. Kirby, J.D. Parker, R. Sadovsky. Cardiovascular Safety of Phosphodiesterase Type 5 Inhibitors After Nearly 2 Decades on the Market. Sex Med Rev. 2018;6:583-594.

[11] M.M. Pauls, B. Moynihan, T.R. Barrick, C. Kruuse, J.B. Madigan, A.H. Hainsworth, J.D. Isaacs. The effect of phosphodiesterase-5 inhibitors on cerebral blood flow in humans: A systematic review. J Cereb Blood Flow Metab. 2018;38:189-203.

[12] A.F. Teich, M. Sakurai, M. Patel, C. Holman, F. Saeed, J. Fiorito, O. Arancio. PDE5 Exists in Human Neurons and is a Viable Therapeutic Target for Neurologic Disease. J Alzheimers Dis. 2016;52:295-302.

[13] E. Vasita, S. Yasmeen, J. Andoh, L.R. Bridges, C. Kruuse, M.M.H. Pauls, A.C. Pereira, A.H. Hainsworth. The cGMP-Degrading Enzyme Phosphodiesterase-5 (PDE5) in Cerebral Small Arteries of Older People. J Neuropathol Exp Neurol. 2019;78:191-194.

[14] S.T. Forgue, B.E. Patterson, A.W. Bedding, C.D. Payne, D.L. Phillips, R.E. Wrishko, M.I. Mitchell. Tadalafil pharmacokinetics in healthy subjects. Br J Clin Pharmacol. 2006;61:280-288.

[15] S.T. Forgue, D.L. Phillips, A.W. Bedding, C.D. Payne, H. Jewell, B.E. Patterson, R.E. Wrishko, M.I. Mitchell. Effects of gender, age, diabetes mellitus and renal and

hepatic impairment on tadalafil pharmacokinetics. Br J Clin Pharmacol. 2007;63:24-35.

[16] A. Garcia-Osta, M. Cuadrado-Tejedor, C. Garcia-Barroso, J. Oyarzabal, R. Franco. Phosphodiesterases as therapeutic targets for Alzheimer's disease. ACS Chem Neurosci. 2012;3:832-844.

[17] C. Garcia-Barroso, A. Ricobaraza, M. Pascual-Lucas, N. Unceta, A.J. Rico, M.A. Goicolea, J. Salles, J.L. Lanciego, J. Oyarzabal, R. Franco, M. Cuadrado-Tejedor, A. Garcia-Osta. Tadalafil crosses the blood-brain barrier and reverses cognitive dysfunction in a mouse model of AD. Neuropharmacology. 2013;64:114-123.

[18] M.M.H. Pauls, N. Clarke, S. Trippier, S. Betteridge, F.A. Howe, U. Khan, C. Kruuse, J.B. Madigan, B. Moynihan, A.C. Pereira, D. Rolfe, E. Rostrup, C.E. Haig, T.R. Barrick, J.D. Isaacs, A.H. Hainsworth. Perfusion by Arterial Spin labelling following Single dose Tadalafil In Small vessel disease (PASTIS): study protocol for a randomised controlled trial. Trials. 2017;18.

[19] L.O. Wahlund, F. Barkhof, F. Fazekas, L. Bronge, M. Augustin, M. Sjogren, A. Wallin, H. Ader, D. Leys, L. Pantoni, F. Pasquier, T. Erkinjuntti, P. Scheltens, C. European Task Force on Age-Related White Matter. A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke. 2001;32:1318-1322.

[20] D.C. Alsop, J.A. Detre, X. Golay, M. Gunther, J. Hendrikse, L. Hernandez-Garcia, H. Lu, B.J. MacIntosh, L.M. Parkes, M. Smits, M.J. van Osch, D.J. Wang, E.C. Wong, G. Zaharchuk. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. Magn Reson Med. 2015;73:102-116.

[21] C.A. Spilling, P.W. Jones, J.W. Dodd, T.R. Barrick. White matter lesions characterise brain involvement in moderate to severe chronic obstructive pulmonary disease, but cerebral atrophy does not. BMC Pulm Med. 2017;17:92.

[22] Y. Zhang, M. Brady, S. Smith. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging. 2001;20:45-57.

[23] C.A. Wauschkuhn, K. Witte, S. Gorbey, B. Lemmer, L. Schilling. Circadian periodicity of cerebral blood flow revealed by laser-Doppler flowmetry in awake rats: relation to blood pressure and activity. Am J Physiol Heart Circ Physiol. 2005;289:H1662-1668.

[24] M. Diamant, M.P. Harms, R.V. Immink, J.J. Van Lieshout, G.A. Van Montfrans. Twenty-four-hour non-invasive monitoring of systemic haemodynamics and cerebral blood flow velocity in healthy humans. Acta Physiol Scand. 2002;175:1-9.

[25] A. Siennicki-Lantz, F. Reinprecht, J. Axelsson, S. Elmstahl. Cerebral perfusion in the elderly with nocturnal blood pressure fall. Eur J Neurol. 2007;14:715-720.

[26] W.B. White, F. Jalil, D.B. Wakefield, R.F. Kaplan, R.W. Bohannon, C.B. Hall, N. Moscufo, D. Fellows, C.R.G. Guttmann, L. Wolfson. Relationships among clinic, home, and ambulatory blood pressures with small vessel disease of the brain and functional status in older people with hypertension. Am Heart J. 2018;205:21-30.

[27] G.L. Schwartz, K.R. Bailey, T. Mosley, D.S. Knopman, C.R. Jack, Jr., V.J. Canzanello, S.T. Turner. Association of ambulatory blood pressure with ischemic brain injury. Hypertension. 2007;49:1228-1234.

[28] M. Guazzi, M. Vicenzi, R. Arena, M.D. Guazzi. PDE5 inhibition with sildenafil improves left ventricular diastolic function, cardiac geometry, and clinical status in

patients with stable systolic heart failure: results of a 1-year, prospective, randomized, placebo-controlled study. Circ Heart Fail. 2011;4:8-17.

[29] F. Murray, M.R. Maclean, P.A. Insel. Role of phosphodiesterases in adult-onset pulmonary arterial hypertension. Handb Exp Pharmacol. 2011:279-305.

[30] M. Lorberboym, I. Mena, J. Wainstein, M. Boaz, Y. Lampl. The effect of sildenafil citrate (Viagra) on cerebral blood flow in patients with cerebrovascular risk factors. Acta Neurol Scand. 2010;121:370-376.

[31] M. Lorberboym, E. Makhline, Y. Lampl. Regional cerebral blood flow following single-dose and continuous-dose tadalafil after stroke. Acta Neurol Scand. 2014;130:380-386.

[32] M. Sheng, H. Lu, P. Liu, Y. Li, H. Ravi, S.L. Peng, R. Diaz-Arrastia, M.D. Devous, K.B. Womack. Sildenafil Improves Vascular and Metabolic Function in Patients with Alzheimer's Disease. J Alzheimers Dis. 2017;60:1351-1364.

[33] S. Jahshan, L. Dayan, G. Jacob. Nitric oxide-sensitive guanylyl cyclase signaling affects CO2-dependent but not pressure-dependent regulation of cerebral blood flow. Am J Physiol Regul Integr Comp Physiol. 2017;312:R948-R955.

[34] C. Kruuse, A.E. Hansen, H.B. Larsson, M. Lauritzen, E. Rostrup. Cerebral haemodynamic response or excitability is not affected by sildenafil. J Cereb Blood Flow Metab. 2009;29:830-839.

[35] U. Lindberg, N. Witting, S.L. Jorgensen, J. Vissing, E. Rostrup, H.B.W. Larsson, C. Kruuse. Effects of Sildenafil on Cerebrovascular Reactivity in Patients with Becker Muscular Dystrophy. Neurotherapeutics. 2017;14:182-190.

[36] J.R. Jennings, M.F. Muldoon, J. Price, I.C. Christie, C.C. Meltzer. Cerebrovascular support for cognitive processing in hypertensive patients is altered by blood pressure treatment. Hypertension. 2008;52:65-71.

[37] M.R. Walters, A. Bolster, A.G. Dyker, K.R. Lees. Effect of perindopril on cerebral and renal perfusion in stroke patients with carotid disease. Stroke. 2001;32:473-478.

[38] F.S. Nazir, J.R. Overell, A. Bolster, T.E. Hilditch, J.L. Reid, K.R. Lees. The effect of losartan on global and focal cerebral perfusion and on renal function in hypertensives in mild early ischaemic stroke. J Hypertens. 2004;22:989-995.

[39] Y. Kimura, K. Kitagawa, N. Oku, K. Kajimoto, H. Kato, M. Tanaka, M. Sakaguchi, H. Hougaku, S. Sakoda, J. Hatazawa. Blood pressure lowering with valsartan is associated with maintenance of cerebral blood flow and cerebral perfusion reserve in hypertensive patients with cerebral small vessel disease. J Stroke Cerebrovasc Dis. 2010;19:85-91.

[40] Y. Kimura, K. Kitagawa, N. Oku, K. Kajimoto, H. Kato, M. Tanaka, M. Sakaguchi, H. Hougaku, S. Sakoda, J. Hatazawa. Hemodynamic influences of azelnidipine, a novel calcium channel blocker, on cerebral circulation in hypertensive patients with ischemic white matter lesions. Hypertens Res. 2008;31:2147-2154.

[41] I.D. Croall, D.J. Tozer, B. Moynihan, U. Khan, J.T. O'Brien, R.G. Morris, V.C. Cambridge, T.R. Barrick, A.M. Blamire, G.A. Ford, H.S. Markus, P.S. Team. Effect of Standard vs Intensive Blood Pressure Control on Cerebral Blood Flow in Small Vessel Disease: The PRESERVE Randomized Clinical Trial. JAMA Neurol. 2018;75:720-727.

[42] D. Tryambake, J. He, M.J. Firbank, J.T. O'Brien, A.M. Blamire, G.A. Ford. Intensive blood pressure lowering increases cerebral blood flow in older subjects with hypertension. Hypertension. 2013;61:1309-1315.

[43] D.L.K. de Jong, R.A.A. de Heus, A. Rijpma, R. Donders, M.G.M. Olde Rikkert, M. Gunther, B.A. Lawlor, M.J.P. van Osch, J. Claassen. Effects of Nilvadipine on

Cerebral Blood Flow in Patients With Alzheimer Disease. Hypertension. 2019;74:413-420.

[44] F.S. Nazir, J.R. Overell, A. Bolster, T.E. Hilditch, K.R. Lees. Effect of perindopril on cerebral and renal perfusion on normotensives in mild early ischaemic stroke: a randomized controlled trial. Cerebrovasc Dis. 2005;19:77-83.

[45] G.W. Blair, J.P. Appleton, K. Flaherty, F. Doubal, N. Sprigg, R. Dooley, C. Richardson, I. Hamilton, Z.K. Law, Y. Shi, M.S. Stringer, M.J. Thrippleton, J. Boyd, K. Shuler, P.M. Bath, J.M. Wardlaw. Tolerability, safety and intermediary pharmacological effects of cilostazol and isosorbide mononitrate, alone and combined, in patients with lacunar ischaemic stroke: The LACunar Intervention-1 (LACI-1) trial, a randomised clinical trial. EClinicalMedicine. 2019;11:34-43.

[46] C. Dufouil, J. Chalmers, O. Coskun, V. Besancon, M.G. Bousser, P. Guillon, S. MacMahon, B. Mazoyer, B. Neal, M. Woodward, N. Tzourio-Mazoyer, C. Tzourio. Effects of blood pressure lowering on cerebral white matter hyperintensities in patients with stroke: the PROGRESS (Perindopril Protection Against Recurrent Stroke Study) Magnetic Resonance Imaging Substudy. Circulation. 2005;112:1644-1650.

[47] J.D. Williamson, N.M. Pajewski, A.P. Auchus, R.N. Bryan, G. Chelune, A.K. Cheung, M.L. Cleveland, L.H. Coker, M.G. Crowe, W.C. Cushman, J.A. Cutler, C. Davatzikos, L. Desiderio, G. Erus, L.J. Fine, S.A. Gaussoin, D. Harris, M.K. Hsieh, K.C. Johnson, P.L. Kimmel, M.K. Tamura, L.J. Launer, A.J. Lerner, C.E. Lewis, J. Martindale-Adams, C.S. Moy, I.M. Nasrallah, L.O. Nichols, S. Oparil, P.K. Ogrocki, M. Rahman, S.R. Rapp, D.M. Reboussin, M.V. Rocco, B.C. Sachs, K.M. Sink, C.H. Still, M.A. Supiano, J.K. Snyder, V.G. Wadley, J. Walker, D.E. Weiner, P.K. Whelton, V.M. Wilson, N. Woolard, J.T. Wright, Jr., C.B. Wright. Effect of Intensive

vs Standard Blood Pressure Control on Probable Dementia: A Randomized Clinical Trial. JAMA. 2019;321:553-561.

[48] I.M. Nasrallah, N.M. Pajewski, A.P. Auchus, G. Chelune, A.K. Cheung, M.L. Cleveland, L.H. Coker, M.G. Crowe, W.C. Cushman, J.A. Cutler, C. Davatzikos, L. Desiderio, J. Doshi, G. Erus, L.J. Fine, S.A. Gaussoin, D. Harris, K.C. Johnson, P.L. Kimmel, M. Kurella Tamura, L.J. Launer, A.J. Lerner, C.E. Lewis, J. Martindale-Adams, C.S. Moy, L.O. Nichols, S. Oparil, P.K. Ogrocki, M. Rahman, S.R. Rapp, D.M. Reboussin, M.V. Rocco, B.C. Sachs, K.M. Sink, C.H. Still, M.A. Supiano, J.K. Snyder, V.G. Wadley, J. Walker, D.E. Weiner, P.K. Whelton, V.M. Wilson, N. Woolard, J.T. Wright, Jr., C.B. Wright, J.D. Williamson, R.N. Bryan. Association of Intensive vs Standard Blood Pressure Control With Cerebral White Matter Lesions. JAMA. 2019;322:524-534.

[49] R. Peters, C.S. Anderson. Advancing dementia prevention through effective blood pressure control. Lancet Neurol. 2020;19:25-27.

Table 1. Participant demographics for the study cohort

	Participants who consented and were randomised (N=65)			Participants who completed the protocol (N=55)		
Variable	All	placebo followed by tadalafil	tadalafil followed by placebo	All	placebo followed by tadalafil	tadalafil followed by placebo
N	65	32	33	55	25	30
Age (y)	66.7 (8.7)	68.0 (8.4)	65.5 (9.0)	66.8 (8.6)	67.9 (8.4)	65.9 (8.9)
Age range (y)	52, 87	53, 87	52, 83	52, 87	53, 87	52, 83
Female/Male	19/46	14/18	5/28	15/40	10/15	5/25
MoCA score	25.4 (3.4)	25.4 (3.3)	25.4 (3.6)	25.1 (3.5)	25.0 (3.4)	25.2 (3.7)
Education (y)	12.8 (3.1)	12.7 (2.9)	12.8 (3.3)	12.7 (3.2)	12.7 (3.0)	12.7 (3.4)
Time from stroke to consent (months)	16.0 (17.6)	15.3 (12.0)	16.8 (22.6)	14.6 (12.1)	14.9 (11.8)	14.3 (12.8)

Modified Rankin score (0/1/2/3/4/5-6)	18/26/16/3/2/0	7/13/10/2/0/0	11/13/6/1/2/0	16/20/14/3/2/0	5/10/8/2/0/0	11/10/6/1/2/0
NIHSS (range 0-42)	1.0 [0.0, 2.0]	1.0 [0.0, 2.0]	1.0 [0.0, 2.0]	1.0 [0.0, 2.0]	1.0 [0.0, 3.0]	0.5 [0.0, 2.0]
WMH volume (mm^3)	NA	NA	NA	14,600 [7,200, 31,700]	15,700 [9,200, 34,500]	11,800 [6,800, 27,600]
Cerebral microbleeds, total count	1 [0, 4]	1 [0, 4]	1 [0, 4]	1 [0, 4]	1.5 [0, 4]	1 [0, 4.5]
SBP (mm Hg)	145 (16.6)	147 (17.1)	144 (16.4)	145 (16.6)	147 (18.7)	144 (14.8)
DBP (mm Hg)	81.0 (10.7)	81.0 (9.6)	81.0 (11.9)	79.9 (10.7)	79.2 (9.7)	80.5 (11.6)

Abbreviations. DBP: diastolic blood pressure; MoCA: Montreal Cognitive Assessment; NIHSS: National Institutes of Health Stroke Scale; SBP systolic blood pressure; WMH: white matter hyperintensities.

Data are reported as mean (SD), except for modified Rankin score (actual scores listed), NIHSS score, WMH volume and cerebral microbleed counts, which are reported as median [inter-quartile range]. Scoring in MoCA ranges from 0 to 30, with a score of 26 or higher indicating normal cognitive ability. These scores have been adjusted for educational level (+1

if the participant had 12 or more years of education). SBP, DBP are the average over visit#1 and visit#2.

Table 2. Effect of placebo, tadalafil and treatment effect on CBF and blood pressure.

Variable	Pre-dose value, Placebo. Mean (SD)	Pre-dose value, Tadalafil. Mean (SD)	Change following Placebo. Mean (95% CI)	Change following Tadalafil. Mean (95% CI)	Treatment effect. Mean (95% CI)
Deep grey matter CBF (ml/min/100g)	24.2 (6.1)	24.5 (7.0)	1.75 (0.74, 2.76) **p=0.0010**	1.79 (0.71, 2.88) **p=0.0016**	0.11 (-1.27, 1.48) p=0.881
Normal appearing white matter CBF (ml/min/100g)	13.5 (4.5)	13.5 (5.2)	0.80 (0.14, 1.47) **p=0.0185**	1.15 (0.49, 1.80) **p=0.0009**	0.33 (-0.54, 1.21) p=0.458
White matter hyperintensities CBF (ml/min/100g)	9.5 (5.6)	9.4 (5.9)	0.32 (-0.48, 1.12) p=0.424	1.29 (0.21, 2.38) **p=0.0203**	0.95 (-0.15, 2.05) p=0.0960
Total grey matter CBF (ml/min/100g)	33.0 (7.8)	33.4 (8.7)	2.05 (0.93, 3.17) **p=0.0006**	2.54 (1.48, 3.61) **p<0.0001**	0.56 (-0.90, 2.02) p=0.456
SBP (mm Hg)	145 (16.0)	145 (15.6)	2.9 (-0.6, 6.4)	-5.0 (-8.6, -1.4)	-7.8 (-11.8, -3.9)

			p=0.107	**p=0.0068**	**p=0.0004**
DBP (mm Hg)	80.6 (8.6)	79.6 (9.4)	0.6 (-1.6, 2.7) p=0.619	-4.0 (-6.0, -2.0) **p=0.0002**	-4.9 (-7.3, -2.4) **p=0.0003**

Abbreviations. CBF: cerebral blood flow; DBP: diastolic blood pressure; SBP systolic blood pressure.

Changes are presented with effect estimate, 95% confidence intervals and associated p-values. SBP and DBP data were derived from post hoc exploratory analyses (not pre-specified endpoints). P-values were not adjusted for age or sex. Analyses were based on 55 participants who completed the protocol. One participant had incomplete placebo CBF data and one other incomplete tadalafil CBF data, hence n=53 for CBF data. For SBP and DBP, n=55.

SUPPLEMENTAL MATERIAL

The PASTIS trial. Testing tadalafil for possible use in vascular cognitive impairment

Mathilde MH Pauls MD[a,b], Lauren R Binnie BSc[a], Philip Benjamin PhD FRCR[a,c], Shai Betteridge[d], Brian Clarke MD[b], Mohani-Preet K Dhillon MRes[a], Rita Ghatala MSc[e], Fearghal AH Hainsworth[a], Franklyn A Howe DPhil[a], Usman Khan PhD[b], Christina Kruuse PhD[f], Jeremy B Madigan MD FRCR[c], Barry Moynihan MD[b,g], Bhavini Patel PhD[b], Anthony C Pereira MD FRCP[a,b], Egill Rostrup DMSc[h], Anan BY Shtaya PhD FRCS[a], Catherine A Spilling PhD[a], Sarah Trippier MSc[e], Rebecca Williams MSc[e], Robin Young PhD[i], Thomas R Barrick PhD[a], Jeremy D Isaacs PhD MRCP[a,b] and Atticus H Hainsworth PhD[a,b] *

[a]Molecular & Clinical Sciences Research Institute, St George's University of London, UK; [b]Department of Neurology, [c]Neuroradiology, [d]Neuropsychology, St George's University Hospitals NHS Foundation Trust, London, UK; [e]South London Stroke Research Network, London, UK; [f]Department of Neurology and Neurovascular Research Unit, Herlev Gentofte Hospital, Denmark; [g]Department of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland; [h]Mental Health Centre, University of Copenhagen, Glostrup, Denmark; [i]Robertson Centre for Biostatistics, University of Glasgow, UK.

*corresponding author: Dr AH Hainsworth PhD, St George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom. tel +44 208 725 5586 fax +44 208 725 2950. email: ahainsworth@sgul.ac.uk

Expanded Materials & Methods

Standard Protocol Approvals, Registrations, and Patient Consents

The trial received ethical approval from the UK National Research Ethics Service, London-Brent Committee on 6th May 2015 (REC reference: 15/LO/0714). Written informed consent was obtained from all participants or their next of kin. The trial was prospectively registered in the European Union Clinical Trials Register (EudraCT number 2015-001235-20; registered 13/05/2015) and in the ClinicalTrials.gov database (NCT02450253; registered 18/05/2015).

Trial design – Randomisation and Treatment

All participants were recruited as part of a phase-II double-blind crossover trial: Perfusion by Arterial spin labelling following Single dose Tadalafil In Small vessel disease (PASTIS)[1]. Participants were enrolled by members of the trial team (MMHP, RG, ST, RW) and randomised to order of treatment (tadalafil 20 mg, placebo; oral administration). The randomisation list was generated in advance by Sharp Clinical Services, Crickhowell, Powys, UK. Randomisation was in blocks as detailed in the Client Study Information form kept in the Sponsor Site File. Random allocation was implemented as sequentially numbered participant packs, each containing two identical child-resistant, tamper-evident bottles, one holding a tadalafil 20mg capsule and one a matched placebo capsule, both over-encapsulated. Each participant received on two separate occasions, visit#1 and visit#2, a placebo dose and a tadalafil 20mg dose which were identical in size, shape, weight and colour.
Two study visits were performed at least 7 days apart, with blood pressure measurement, MRI scanning and a battery of cognitive tests up to 3 h before and 3-5 h after dosing (see Fig.1A). Participants, care providers and those assessing outcomes were all blind to treatment allocation.

Trial Endpoints

The primary endpoint was change in CBF in subcortical brain tissue. This was assessed in three tissue types: DGM, NAWM and WMH. Change in CBF for cortical grey matter was a secondary endpoint.

Power analyses

We aimed to detect a treatment effect of at least 15% in subcortical CBF, with 90% statistical power using a two-tailed paired t-test at the 0.05 significance level. We assumed average CBF (± SD) of 70 (±15) ml/100g/min in grey matter and 30 (±10) ml/100g/min in subcortical white matter. We estimated that a sample size of N=24 would be required in grey matter and N=54 in white matter[1].

Setting of the Study

The trial commenced on 4th September 2015. Participants were recruited from St George's University Hospitals NHS Foundation Trust and local Participant Identification Centre sites. All patient visits, data management and trial coordination were performed at the St George's site. PASTIS was adopted onto the UK NIHR Clinical Research Network Portfolio (CRN Study ID# 18978). The trial ended when the pre-determined recruitment target was met (25 January 2018).

Study population

All data were from a cohort of older adults without known diagnosis of dementia, with radiological and clinical evidence of symptomatic SVD. Demographic details are summarized in Table 1. Inclusion criteria were as follows. 1, radiological evidence of SVD, defined as: MRI evidence of lacunar infarct(s) up to 15 mm maximum diameter and/or confluent deep WMH (grade 2 or higher on the Fazekas scale[2]). 2, clinical evidence of cerebral small vessel disease defined as either: lacunar stroke syndrome with symptoms lasting at least 24 hours, occurring at least 6 months prior to visit#1; or: transient ischaemic attack lasting less than 24 hours with limb weakness, hemi-sensory loss or dysarthria at least 6 months previously and with diffusion-weighted MRI performed acutely showing lacunar infarction. If MRI was not performed within 10 days of TIA, a lacunar infarction in an anatomically appropriate position as demonstrated on a subsequent MRI was also deemed eligible. 3, Age at least 50 years. 4, imaging of the carotid arteries with Doppler ultrasound, CT angiography or MR angiography in the previous 12 months, demonstrating less than 70% stenosis in both internal carotid arteries or less than 50% stenosis in both internal carotids if measured in previous 12-60 months. Exclusion criteria included: known diagnosis of dementia; cortical infarction (more than 15 mm maximum width); systolic BP below 90 mmHg; diastolic BP below 50 mmHg; creatinine clearance less than 30ml/min; stroke or TIA within the previous 6 months; concomitant use of PDE5i. A full list of exclusion criteria is given in the published protocol[1].

Clinical Assessments

Participants attended an initial screening visit ("Visit 0") and completed an eligibility check. Informed consent was documented. During the screening visit, education level and Montreal Cognitive Assessment (MoCA) scores were recorded (Table 1). Following consent, participants attended two study visits (Visit#1, Visit#2) at least 7 days apart [1]. At each study visit, participants underwent systolic/diastolic blood pressure (SBP/DBP) measurements, a cognitive test battery[1] and brain MRI scanning. SBP/DBP measurements were taken from each participant for each visit, first on arrival after resting, then again after MRI scanning, using a validated Omron MX3Plus machine.

Changes to Methods after trial commencement

Amendments were made to the published protocol[1]:

- to perform some cognitive testing in Visit 0 (from 02 September 2015).
- Eligibility criteria were adjusted to allow lower age limit of 50 and lower Creatinine Clearance of 30ml/min (from 29 October 2015).

Blood sampling and analyses

At the end of each study visit, and at least 3 h post dosing, a blood sample (approximately 5 ml) was taken for full blood count. A second blood sample was taken (5 ml) for subsequent analysis of tadalafil concentration. Blood was taken in purple capped EDTA tubes, inverted to mix, and centrifuged at room temperature at 3000 RPM for 5 minutes to remove cellular material. Plasma (approximately 1.5-2.0 ml) was decanted into a labelled plastic cryovial, then transferred to a designated -80 ^{o}C freezer. Plasma tadalafil concentration was measured by LC-MS-MS assay (ASI Bioanalytics Ltd, London UK, https://www.bioanalytics.co.uk/).

The trial was subject to an ICH-Good Clinical Practice inspection by the Medicines and Healthcare Products Regulatory Agency (MHRA), the statutory regulator in the UK in

September 2019 which identified breaches of ICH-GCP associated with sample analysis. The analytical method used was a forensic toxicology procedure rather than a method which had been validated against the European Medicines Agency Bioanalytical Method Validation guidance. Though tadalafil has high freeze/thaw stability [3] the impact of the storage of samples and freeze/thaw cycles, along with other assessments stated in the European Medicines Agency guidance, on tadalafil plasma concentration were not determined during this trial. All tadalafil concentrations reported here were derived from first analysis, so were not subject to repeated freeze/thaw effects. The maximum duration between sample storage at -80 ºC and analysis was 892 days (median 454 days, IQR 366-586 days).

An additional ICH-GCP breach identified that plasma tadalafil levels were analysed prematurely in fifteen participants, resulting in the chief investigator (AHH) being unblinded to the treatment group for these individuals. The trial had been designed as a double-blinded randomized control trial which meant that no members of the research team should have been aware of IMP regime of any of the subjects during the trial. As the chief investigator had no direct role in patient assessment or data acquisition and performed none of the data analyses reported, this was not considered to have compromised the trial outcomes or conclusions and the study continues to remain double-blinded (i.e. patient-blinded and clinician-blinded).

Magnetic Resonance Image Acquisition

Whole-brain perfusion MRI was acquired using a 3T scanner (Achieva Dual TX MRI scanner, Philips Medical Systems, Eindhoven, Netherlands) at St George's University Hospitals NHS Foundation Trust. Whole brain T1-weighted, Fluid Attenuated Inversion Recovery (FLAIR), susceptibility-weighted imaging (SWI) and pseudo-continuous arterial spin labelling (pCASL) images (which included a proton-density weighted image) were acquired. All MRI data were acquired from brain scans performed on a Tuesday or Thursday, pre-dosing scans between the hours of 10:00 and 12:00 and post-dosing scans 14.00-17.00.

T1-weighted MRI. Whole brain sagittal 3D T1-weighted images were acquired to enable tissue segmentation with the following protocol: Turbo Field Echo (TFE) sequence with an inversion pre-pulse, TFE factor 240 in multi-shot mode with 3000 ms shot interval, 8° flip angle, TR 7.9 ms, TE 3.8 ms, 1mm×1mm×1.5mm acquired resolution with interpolation to 1 mm isotropic resolution, 1 average and SENSE factor 2 for a 3 minutes 47 seconds acquisition time.

FLAIR MRI. 2D T2-weighted axial FLAIR images were acquired to detect WMH using the following protocol: T2 weighted turbo-spin-echo sequences with selective fat suppression (TSE-SPIR), TR 11000ms, TE 120ms, TI 2800ms, 0.65mm×1.00mm acquired resolution interpolated to 0.45×0.45 mm over 24 thick slices (5 mm thickness), with 2 averages and a 1.75 SENSE factor for a 3 minutes 51 seconds acquisition time.

pCASL MRI. Our pCASL protocol was developed based on the consensus recommendations of the ISMRM Perfusion study group and European consortium for ASL in dementia[4] using the Philips product pCASL sequence in the scanner 5.3 software release. A 64×64 acquisition matrix with 16 slices was used to acquire data with 4 x 4 mm in-plane resolution with 6mm slice thickness and 1 mm slice gap (hence, approximate voxel size 4mm×4mm×6mm). Background tissue suppression and SPIR fat suppression were applied to improve the contrast to noise of the blood perfusion signal. A total of 140 volumes (alternating with and without the spin labelling inversion pulse) were acquired in two separate 10 minute acquisitions using SENSE 2.3, and TE 8ms and TR 4300ms with a labelling duration (τ) = 1800ms and post labelling delay = 2000ms. A fixed labelling distance of 85 mm from the centre of the imaging block was used with the labelling slice positioned below the cerebellum

at an angle perpendicular to the carotid arteries (visualized by time of flight angiography). Proton density weighted images were acquired using the pCASL sequence without the inversion pulse and background suppression, but with fat suppression and an increased TR 5000ms to reduce T1 weighting effects (TE 9ms with 8 averages). Supplemental Fig. S1 provides an outline of the MRI data analysis pipeline.

Computation of CBF maps

The pCASL data acquisitions at each visit were corrected for subject movement using the FMRIB software library function *eddy_correct* (fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL). An average pCASL map was then separately computed for each pCASL data acquisition. The average pCASL maps and the second proton density weighted image were aligned to the initial proton density weighted image in each scan session using the FSL Linear Image Registration Tool (flirt) [5]. These transformations were applied to the motion-corrected pCASL data to ensure all proton density weighted and pCASL images were aligned in the same space. The aligned proton density weighted images were averaged and CBF was computed using *oxford_asl* (part of the FSL-BASIL toolset, fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL). Cerebral blood flow in each voxel was calculated using the standard equation for pCASL[4]:

$$CBF = \frac{6000 \cdot \lambda \cdot (SI_{control} - SI_{label}) \cdot exp\left(\frac{PLD}{T1_{blood}}\right)}{2 \cdot \alpha \cdot T1_{blood} \cdot SI_{PD} \cdot \left(1 - exp\left(-\frac{\tau}{T1_{blood}}\right)\right)} \quad \text{ml/100g/min} \qquad \text{(Equation 1)}$$

where $SI_{control}$ and SI_{label} are the time-averaged signal intensities in the pCASL control and label images, respectively, and SI_{PD} is the signal intensity of a proton density weighted image. Standard values were inserted into Equation 1 for the brain/blood partition coefficient, λ=0.9 ml/g, the labeling efficiency, τ=0.85, longitudinal relaxation time of arterial blood $T_{1,blood}$=1650 ms at 3T. An example of a pCASL map is shown in Fig.2.

WMH delineation

WMHs were delineated on each axial slice of the Visit#1 FLAIR images using commercially available JIM software v7.0 (Xinapse Systems Ltd, West Bergholt, Essex, UK). WMH were defined as hyperintense regions, which were (1) not due to presence of blood vessels and (2) not less than 10 mm^2 in size and (3) not a narrow band, one pixel wide, along the edge of the ventricles. A binary WMH image was generated and the total WMH volume (in mm^3) was computed for each participant. All WMH maps used here were produced by a single operator, blind to treatment allocation and to all clinical details (FAHH). A second, blinded operator (MMHP) independently produced maps for a subset of participants (n=51) and inter-operator agreement was good (WMH volume Intraclass Correlation Coefficient 0.855 [95% CI: 0.760, 0.915]).

Tissue Segmentation

For each scanning session, T1-weighted images in native space were segmented into grey matter, white matter and cerebrospinal fluid (CSF) tissue probability maps (Fig.2). This was performed using a modified form of the standard Statistical Parametric Mapping (SPM Version 12, https://www.fil.ion.ucl.ac.uk/spm/) geodesic shooting segmentation and normalization procedure, described in our previous paper[6]. This procedure captures

population-specific features (e.g. enlarged ventricles) and allows superior delineation of deep grey matter structures compared to the standard SPM pipeline. The binary WMH mask derived from JIM software was co-registered into native T1-weighted space so as to repair the tissue probability maps for any misclassification caused by WMHs.

Native space T1-weighted and native space FLAIR images were skull-stripped using the brain extraction tool within FMRIB software library (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET) then co-registered to the average proton density-weighted image by a process of boundary-based registration (using FSL epi-reg). These 12-parameter linear transformations were used to align the corrected T1-weighted tissue probability maps and the binary WMH map to the CBF maps. A tissue mask in the average proton density-weighted image space was computed. Each voxel in the CBF map was provisionally assigned to either grey matter, normal appearing white matter, WMH or CSF, based on the maximum tissue probability.

Computation of CBF in whole brain tissue

For the alignment of the T1-weighted tissue segmentation images to the low-resolution pCASL images it was necessary to apply a further segmentation step. This tissue segmentation procedure has not been previously applied to ASL data and employs a novel application of a tissue segmentation algorithm to CBF maps[7]. It is designed to assign voxels with high CBF values to grey matter and low CBF values to white matter segments. The distribution of CBF values within the grey matter and white matter tissue masks computed in the Tissue Segmentation section (above) were entered as empirical priors to a Hidden Markov random field model and segmentation (FMRIB's Automated Segmentation Tool, FAST)[7] to provide an improved segmentation of grey and white matter tissue from the CBF maps. This technique reduces the effects of partial volume and tissue classification errors at the boundary between grey and white matter tissue caused by the large pCASL image voxel size and the relative difference between voxel sizes of the native pCASL and T1-weighted images. In particular, this method assigns voxels with high CBF values at the grey/white matter tissue boundary to the grey matter segment and voxels with low CBF values at the grey/white matter tissue boundary to the white matter segment. To avoid misclassification of CSF and WMH regions, voxels in these regions were not entered into the FAST segmentation step. For each participant at each scan session, median CBF values were calculated for total grey matter, NAWM and WMH (example in Fig.2).

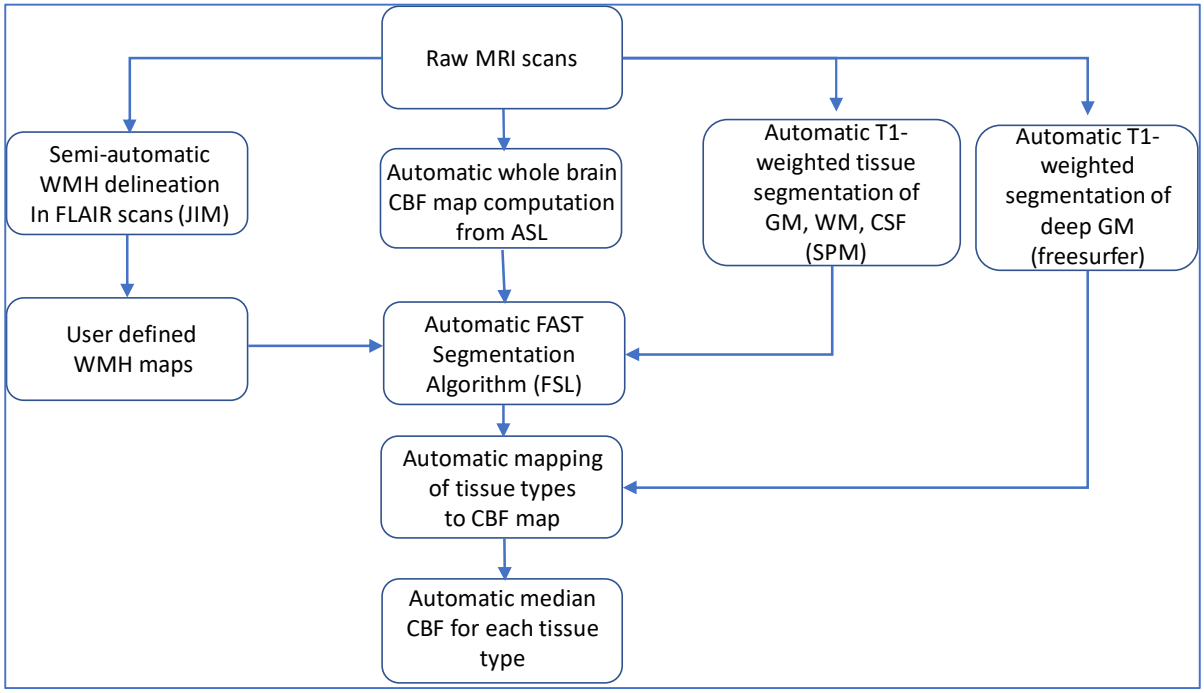
Computation of CBF in deep grey matter structures

Cerebral deep grey matter structures were segmented on native space T1-weighted images using Freesurfer (Freesurfer Version 5.3.0, https://surfer.nmr.mgh.harvard.edu/fswiki/). The binary segmentations of the caudate, putamen and thalamus were aligned to the CBF maps by application of the affine transformation computed in Tissue Segmentation (see above). Median CBF values were calculated for all voxels in each of these three anatomical deep grey matter structures across the left and right cerebral hemispheres. The average of these three median values is reported for CBF in deep grey matter (DGM).

Counting cerebral microbleeds in susceptibility weighted scans

All participants were assessed for the presence of cerebral microbleeds (CMBs) using recognised criteria, the BOMBS rating protocol (Cordonnier et al. 2009). Susceptibility

weighted (SW) scans were examined visually. CMBs were defined as hypointense foci up to 10 mm in greatest diameter. All microbleed assessments were performed by a single experienced observer (Mr A Shtaya FRCS). SW scans were available for 60 participants, among whom 40 (67%) had at least 1 CMB, 11 (18%) had only 1 CMB, 12 (20%) had 2-3 CMBs and 17 (28%) had more than 3 CMBs (range 4-49).



Supplementary Figure S1. MRI data analysis workflow.

FAST, JIM, SPM and freesurfer are all software packages. Most processes are automated. The exception is semi-automatic WMH delineation using JIM software, to produce user-defined WMH maps.

Abbreviations. ASL: arterial spin labelling; CBF: cerebral blood flow; CSF: cerebrospinal fluid; FLAIR: Fluid Attenuated Inversion Recovery; FMRIB: Functional Magnetic Resonance Imaging of the Brain; FSL: FMRIB software library; GM: grey matter; WM: white matter; WMH: white matter hyperintensities.

Supplemental Table S1. Table of Adverse Events in the PASTIS trial

Partici-pant No.	Age (y)	Sex	Group 1 or 2	Treatment at onset of AE	Duration of AE	Description of AE
6	75	f	2	Placebo	2 d	Headache and vomited after visit 1 at home; had passed by the next morning. Withdrew from the trial.
13	72	m	2	Placebo	2 d	Had a cold and did not tolerate first MRI scan, study visit abandoned.
15	61	f	1	Placebo	1 day	Had headache lasting 2 mins after lunch.
17	69	f	2	Placebo	1 day	Diabetes mellitus type 1 (all adult life); had a hypoglycaemic event during visit 1. Resolved after a sugary drink and fruit.
22	77	f	1	Placebo	6 days	Had a chest infection treated by GP with antibiotics between visits 1 and 2.
33	59	f	2	Tadalafil	5 days	COPD Asthma (lifelong); had lower respiratory tract infection.
33	59	f	2	Tadalafil	3 days	Left knee pain (psoriatric arthritis).
37	73	f	1	Placebo	11 days	Sore throat and cough and feeling unwell. Cancelled visit 2 due to inability to lie still with cough. Re-enrolled as #51.
41	56	m	2	Placebo	1 day	Felt flushed and slightly faint for 5 mins, starting about 30 mins after treatment. Recovered spontaneously and felt better after a few hours.
49	57	m	2	Placebo	1 day	Panic attack in MRI scanner during visit 1. Withdrew from the trial.
59	72	f	1	Tadalafil	1 day	Felt lightheaded after first MRI scan on visit 1. Had a sandwich and felt better in 10 mins.

Abbreviations: AE: adverse event. Note that two AEs relate to the same participant (#33).

Supplemental References

[1] M.M.H. Pauls, N. Clarke, S. Trippier, S. Betteridge, F.A. Howe, U. Khan, C. Kruuse, J.B. Madigan, B. Moynihan, A.C. Pereira, D. Rolfe, E. Rostrup, C.E. Haig, T.R. Barrick, J.D. Isaacs, A.H. Hainsworth. Perfusion by Arterial Spin labelling following Single dose Tadalafil In Small vessel disease (PASTIS): study protocol for a randomised controlled trial. Trials. 2017;18.

[2] L.O. Wahlund, F. Barkhof, F. Fazekas, L. Bronge, M. Augustin, M. Sjogren, A. Wallin, H. Ader, D. Leys, L. Pantoni, F. Pasquier, T. Erkinjuntti, P. Scheltens, C. European Task Force on Age-Related White Matter. A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke. 2001;32:1318-1322.

[3] K.Y. Rust, H. Wilkens, R. Kaiser, D. Bregel, J. Wilske, T. Kraemer. Detection and validated quantification of the phosphodiesterase type 5 inhibitors sildenafil, vardenafil, tadalafil, and 2 of their metabolites in human blood plasma by LC-MS/MS--application to forensic and therapeutic drug monitoring cases. Ther Drug Monit. 2012;34:729-735.

[4] D.C. Alsop, J.A. Detre, X. Golay, M. Gunther, J. Hendrikse, L. Hernandez-Garcia, H. Lu, B.J. MacIntosh, L.M. Parkes, M. Smits, M.J. van Osch, D.J. Wang, E.C. Wong, G. Zaharchuk. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. Magn Reson Med. 2015;73:102-116.

[5] M. Jenkinson, S. Smith. A global optimisation method for robust affine registration of brain images. Med Image Anal. 2001;5:143-156.

[6] C.A. Spilling, P.W. Jones, J.W. Dodd, T.R. Barrick. White matter lesions characterise brain involvement in moderate to severe chronic obstructive pulmonary disease, but cerebral atrophy does not. BMC Pulm Med. 2017;17:92.

[7] Y. Zhang, M. Brady, S. Smith. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging. 2001;20:45-57.