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Effects of Sapropterin on Endothelium-Dependent Vasodilation in Patients With CADASIL
A Randomized Controlled Trial

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Background and Purpose—Cerebral autosomal–dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a rare autosomal dominant disorder caused by *NOTCH3* mutations, is characterized by vascular smooth muscle and endothelial cells abnormalities, altered vasoreactivity, and recurrent lacunar infarcts. Vasomotor function may represent a key factor for disease progression. Tetrahydrobiopterin, essential cofactor for nitric oxide synthesis in endothelial cells, ameliorates endothelial function. We assessed whether supplementation with sapropterin, a synthetic tetrahydrobiopterin analog, improves endothelium-dependent vasodilation in CADASIL patients.

Methods—In a 24-month, multicenter randomized, double-blind, placebo-controlled trial, CADASIL patients aged 30 to 65 years were randomly assigned to receive placebo or sapropterin 200 to 400 mg BID. The primary end point was change in the reactive hyperemia index by peripheral arterial tonometry at 24 months. We also assessed the safety and tolerability of sapropterin. Analysis was done by intention-to-treat.

Results—The intention-to-treat population included 61 patients. We found no significant difference between sapropterin (n=32) and placebo (n=29) in the primary end point (mean difference in reactive hyperemia index by peripheral arterial tonometry changes 0.19 [95% confidence interval, −0.18, 0.56]). Reactive hyperemia index by peripheral arterial tonometry increased after 24 months in 37% of patients on sapropterin and in 28% on placebo; however, after adjustment for age, sex, and clinical characteristics, improvement was not associated with treatment arm. The proportion of patients with adverse events was similar on sapropterin and on placebo (50% versus 48.3%); serious adverse events occurred in 6.3% versus 13.8%, respectively.

Conclusions—Sapropterin was safe and well-tolerated at the average dose of 5 mg/kg/day, but did not affect endothelium-dependent vasodilation in CADASIL patients.

Clinical Trial Registration—URL: https://www.clinicaltrialsregister.eu. Unique identifier: 2007-004370-55.
(***Stroke***. 2014;45:2959-2966.)

Key Words: CADASIL ■ endothelium ■ nitric oxide ■ randomized controlled trial ■ tetrahydrobiopterin ■ vascular

CADASIL (cerebral autosomal–dominant arteriopathy with subcortical infarcts and leukoencephalopathy), the most common heritable cause of stroke and vascular dementia in adults, is a rare autosomal–dominant disorder caused by mutations in the 33-exon *NOTCH3* gene on chromosome 19p13.1.[1–5] Clinically, CADASIL is characterized by recurrent ischemic events starting in mid-adulthood, with an average incidence rate of stroke of 10.4/100 person-years.[3] Although

extent and mode of disease progression vary largely, many patients with CADASIL eventually develop severe motor disability, pseudobulbar palsy, and subcortical dementia. Treatment of CADASIL is largely empirical at present and based on current cerebrovascular disease management.[1]

NOTCH3 encodes for a transmembrane receptor of a signaling pathway that exerts a central role in vascular development and is prevalently expressed in vascular smooth muscle cells and capillary pericytes in the adult brain.[5] Vasomotor function may play a fundamental role for the eventual development of ischemic lesions. In transgenic mice expressing mutant *NOTCH3* in vascular smooth muscle cells, impaired cerebral blood flow autoregulation and increased myogenic tone predate the appearance of vascular abnormalities,[6] whereas in patients, cerebral blood flow and metabolism are decreased early in the clinical course of CADASIL.[7,8] Structural abnormalities in CADASIL patients encompass lumen narrowing of the arterioles, destruction of vascular smooth muscle cell anchorage to the extracellular matrix, and alterations in the cytoskeleton and in the endothelial cells.[9,10] Moreover, capillaries, which have a major role in blood flow regulation in the healthy and ischemic brain,[11] exhibit pericytes with swollen nuclei and deposits of granular osmiophilic material within the basement membrane between the pericytes and endothelial cells[12]; accumulation of mutated NOTCH3 proteins in pericyte membranes may also alter the function of the endothelial lining

Endothelial nitric oxide (NO) has a crucial role in the cerebral circulation linking cerebrovascular function with cognition.[13] NO is synthesized in the endothelium in a process that involves the enzyme endothelial NO synthase (eNOS), the substrate arginine, and the essential enzyme cofactor tetrahydrobiopterin (BH4).[14] Increased NO bioavailability through eNOS activation preserves cerebral blood flow, prevents inflammation, platelet aggregation, thrombosis, and apoptosis.[13] NO has been suggested to exert paracrine effects on neuronal and microglial cells and plays a critical role in mediating dilation of brain capillaries by affecting active pericyte relaxation.[11] Stabilization of eNOS activity, by enhancing its substrate, cofactors, and transcription, improves NO bioavailability and endothelial function. Decreased BH4 bioavailability results in functional NOS uncoupling,[15,16] impaired vascular relaxation, and endothelial dysfunction (ED). ED occurs in the cerebral circulation in association with vascular-related diseases that include stroke and Alzheimer's disease and has been correlated with increased risk of acute ischemic stroke.[17]

Flow-mediated dilation, the response of the endothelium to shear stress evoked by reactive hyperemia (RH), is a useful test to detect abnormalities in NO production in the vascular wall using different techniques.[18,19] Patients with lacunar infarcts show impaired flow-mediated dilation by brachial artery ultrasound,[20] a measure of conduit artery vasodilation.

Previous studies in CADASIL patients described impaired vasoreactivity both in the cerebral[21,22] and peripheral circulation.[23–26] However ED was shown in resistance arteries, but not in conduit vessels.[24] Flow perturbance by mutated NOTCH3 proteins in capillary pericytes may largely mediate impaired vasoreactivity and pathophysiology of brain damage in CADASIL. Therefore, a method that tests microvascular, rather than conduit artery, reactivity is appropriate to investigate treatment effects in this population.

Peripheral arterial tonometry (PAT), a novel noninvasive, quantitative, and repeatable test that measures changes in digital pulse volume during RH, evaluates the endothelial function of resistance arteries and NO-mediated changes in microvascular response. PAT accurately assesses variations with increasing risk factor burden and targeted treatment[27,28] and is independently associated with incident cardiovascular (CV) events in high risk patients.[29]

An impaired RH-PAT (reactive hyperemia index by peripheral arterial tonometry) response has been observed early after acute cerebrovascular events, including stroke[30] and subarachnoid hemorrhage,[31] and was found to be independently associated with fewer delirium/coma-free days in patients with critical illness.[32] RH-PAT has been shown to be sensitive to treatment effects in patients with diabetes mellitus and coronary artery disease.[33,34]

In a small correlative study, both cerebral blood flow and peripheral vasodilation by RH-PAT were reduced in CADASIL patients.[35] As NO has a fundamental role in cerebral blood flow regulation and small vessel damage, with impaired endothelial integrity, is the pathological hallmark of CADASIL, amelioration of endothelial function is an end point relevant to disease pathophysiology. Exogenous BH4 administration can improve vascular NO bioavailability by reversing eNOS uncoupling and was shown to acutely restore endothelial function in patients with CV risk factors or overt coronary artery disease.[36]

Aim of the present study was to assess whether chronic administration of the BH4 analog sapropterin (6R-BH4) improves endothelium-dependent vasodilation in CADASIL patients.

Methods

Participants

Adult patients aged between 30 and 65 years, with CADASIL with or without previous neurological symptoms or cerebrovascular events, were eligible for screening. The diagnosis had to be confirmed by the identification of a *NOTCH3* gene mutation. Patients were required to have a recent (within 6 months) cranial MRI consistent with CADASIL, which was done for study purposes if not already available.

Exclusion criteria were dementia with a score <33 at the Structured Interview for the Diagnosis of Dementia instrument; autoimmune disorders; pregnancy, nursing or childbearing potential not on adequate contraception; recent (within 3 months before screening) myocardial infarction, cerebrovascular accident, or pulmonary embolism; severe uncontrolled hypertension (arterial blood pressure [BP] >180/110 mm Hg); hypotension at screening, defined as seated resting values of <100 mm Hg systolic or <55 mm Hg diastolic, or symptomatic hypotension; serum creatinine >2.5 mg/dL or hepatic enzymes >2× normal; and concomitant treatment with methotrexate, levodopa, phosphodiesterase-3 or -5 inhibitors, pentoxyfylline, nitrate/nitrite-based vasodilators, L-arginine, or gingko biloba.

Patients were recruited, after providing written informed consent, at 5 national referral centers for CADASIL management. The study was done according to the Declaration of Helsinki and subsequent revisions. The study is registered under the EudraCT number 2007-004370-55 and was approved by the ethics committees of each participating center.

Randomization and Masking

Treatment allocation was managed by a logistic manager at the coordinating center using a computer-generated, 1:1 randomization scheme with a sequential block size of 4 for each clinical center and assigning a randomization code to each subject after screening and confirmation of eligibility. The trial drug or matching placebo was

packed in identical masked bottles marked with the patient's identification number. Patients, their families, the investigators, the study manager, and statistician were masked to treatment assignment.

Procedures

This was a multicenter, phase II, randomized, double-blind, parallel-group, placebo-controlled trial planned to include 60 subjects. Consenting patients entered a 3-month run-in phase to confirm stability on optimized concomitant treatment. Baseline evaluation included history, clinical and neurological evaluation, determination of the modified National Institutes of Health Stroke Scale (mNIHSS) to assess neurological status and the Barthel index and the modified Rankin scale to assess disability, blood sampling for routine laboratory tests, and a vasoreactivity study.

Patients received sapropterin dihydrochloride, a pharmaceutical formulation of BH4, provided in 100 mg fast-dissolving tablets or matching placebo for 24 months. The drug was given in doses of 200 or 400 mg BID, according to body weight ≤ or >60 kg, corresponding to ≈5 mg/kg/day, and were selected based on previous studies of BH4 in subjects with ED and CV risk factors or diseases. Patients were instructed to dissolve tablets in water and take them with food twice a day.

After 1, 2, 4, and 6 months, and every 6 months thereafter, a clinical assessment was performed and blood was sampled for safety laboratory measurements (complete blood count, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, serum bilirubin, and creatinine). After 24 months, baseline assessments were repeated. Treatment compliance was calculated from tablet count for each interval between visits as the percentage of the dispensed study medication taken by the patient.

Study End Points

The primary efficacy end point was the change in endothelium-dependent vasodilation as assessed by RH-PAT after 24-month double-blind treatment with sapropterin or placebo. Secondary efficacy end points included changes in neurological status by the modified National Institutes of Health Stroke Scale and changes in the disability scores Barthel index and modified Rankin scale.

Safety measurements included adverse events, vital signs, and laboratory profile.

Assessment of Endothelial Function

Vasoreactivity studies were performed at the 5 clinical centers by 2 trained investigators. Vasodilation after RH was assessed in the morning in the fasting state using the Endo-PAT 2000 appliance (Itamar, Cesarea, Israel).[28,37] The patient lied down comfortably with hands supported at approximately heart level in room with dim lights and controlled 24°C temperature. A cuff was placed on the nondominant upper arm, whereas the contralateral served as control. BP and heart rate were measured before starting the test from the patient's control arm. After a 10-minute equilibration period, the cuff was inflated to 60 mm Hg above systolic pressure for 5 minutes and then deflated to induce RH, whereas signal recording was continued for 10 minutes. RH-PAT was automatically calculated as operator-independent ratio of the average post- and preocclusion amplitude of the signal, normalized by the control arm.

To exclude an interday variability and to ensure within-subject repeatability, basal levels of RH-PAT were measured in the morning in a group of fasted volunteers (n=9), on 2 consecutive days. Consistently with previous reports,[38] average data obtained did not demonstrate an interday effect on vascular function (RH-PAT 2.05±0.29 for day 1 and 2.04±0.27 for day 2; P=0.94, coefficient of variation 4.67%).

No vasoreactivity study was considered inadequate after blinded assessment before database locking.

Statistical Analysis

The number of patients per group needed to detect an absolute difference between active treatment and placebo after 24 months in RH-PAT of 1.8 with a power of 90%, with a 2-tailed t test at the 5% level and standard deviation of 2 was calculated to be 26. To account for a 15% drop-out rate, 61 patients were randomized.

All analyses were done in 2 study populations: the intention-to-treat (ITT) and the per-protocol population (PP). The ITT population included all patients randomly assigned to treatment and assessed using the last-observation-carried-forward method; as sensitivity analysis, we also imputed data that were missing at follow-up using the multiple imputation method, created 5 complete data sets, analyzed each data set, and pooled the results. The PP population included patients who did not discontinue treatment until the 24-month visit and had a treatment compliance ≥80%.

Continuous variables are presented as median (interquartile range) and categorical variables as numbers and percentages. For categorical data, groups were compared by contingency tables with the χ^2 or Fisher exact test, where appropriate. Continuous data were analyzed using a 2-sided Student's t test, after checking data normal distribution (based on the Shapiro–Wilk statistic) and a 2-sided Wilcoxon's rank sum test otherwise.

To analyze differences between treatment arms in terms of improved endothelial function (change from baseline in RH-PAT at end of study >0), odds ratios and the corresponding 95% confidence intervals (CI) were obtained using unconditional multiple logistic regression, adjusted for prespecified subgroups of interest based on age, sex, presence of any CV risk factors, history of cerebrovascular events, duration of symptoms, and concomitant treatment with drugs active on endothelial function.

All statistical analyses were done with significance set at the 5% level and using 2-sided tests or 2-sided 95% CI, using SAS version 9.1 (Cary, NC, USA).

Results

Patients were enrolled from January 3, 2008, till March 20, 2009; follow-up was completed on April 2011. The number of subjects enrolled and their fate in the study are shown in Figure. Sixty-one subjects from 38 different families were ultimately randomized, 32 to sapropterin and 29 to placebo, and are included in the ITT population. Six of the randomized patients dropped out at different times during the study (Figure); in no case was the study code broken. Overall, 50 patients, 29 in the sapropterin and 21 in the placebo arm, completed the study with a treatment compliance ≥80% and were included in the PP analysis.

Demographics, neurological history, concomitant drug therapy, and other baseline characteristics of the ITT population are shown in Table 1. Despite randomization, male sex and risk factors were more common in the placebo group; however, BP, lipid, and glucose profile overlapped between groups. Treatment with drugs known to favorably affect endothelial function (calcium channel blockers, rennin–angiotensin system inhibitors, or statins) was common, but the overall proportion of subjects who took ≥1 of these drugs was similar between treatment arms. Disability was mild in most subjects, with patients on sapropterin showing lower scores than those on placebo. Overall, 20 subjects (33%), 10 in each treatment arm, showed baseline RH-PAT values below the 5th percentile of a normal control group, indicative of established ED.

We found no significant difference in the study primary end point, RH-PAT change from baseline at 24 months, between patients assigned sapropterin and those assigned placebo either in the ITT or in the PP population (Table 2). In both data sets, changes in RH-PAT between baseline and end of treatment did not achieve statistical significance either in subjects in the sapropterin arm (ITT, P=0.67; PP, P=0.45) or in those on placebo (ITT, P=0.29; PP, P=0.18; Table 2). Among patients who
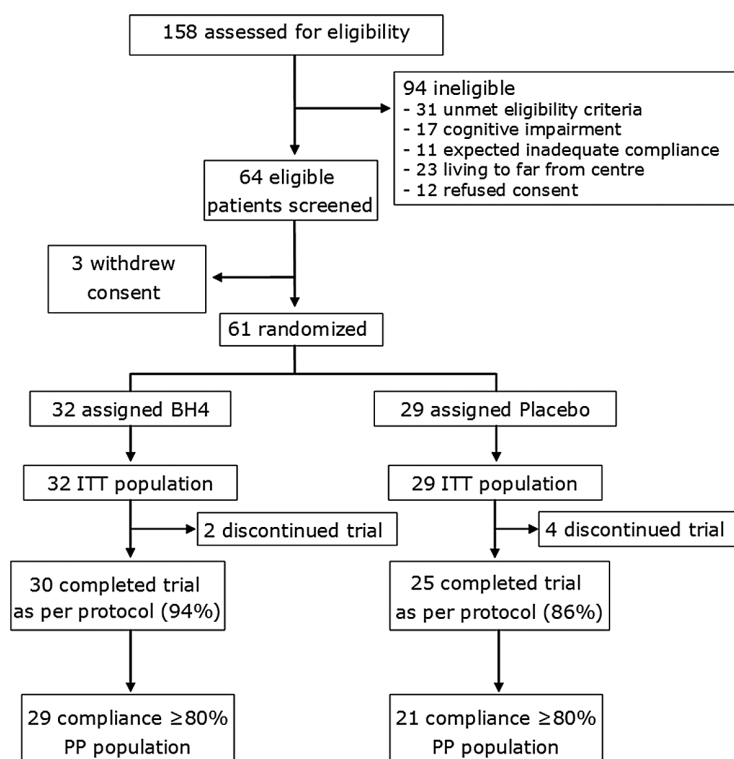
completed the study, RH-PAT values indicative of ED were found in 30% in the sapropterin and 65% in the placebo arm.

The proportion of patients with worsened neurological status or disability scores, expressed as any increase in modified National Institutes of Health Stroke Scale and modified Rankin scale scores or any decrease in Barthel index, did not differ between treatment arms either in the ITT or in the PP population (Table 3).

RH-PAT increased after 24 months in 37% of subjects on sapropterin versus 28% of patients on placebo. By multivariable logistic regression (Table 4), after adjustment for demographic and clinical characteristics, no association was observed between treatment arm and improved RH-PAT (odds ratio, 2.42; 95% CI, 0.72, 8.14).

The proportion of patients with treatment-emergent adverse events was similar between groups (50% on sapropterin versus 48.3% on placebo; Table 5). No death occurred. Serious adverse events were reported in 2 (6.3%) patients assigned to sapropterin and in 4 (13.8%) patients assigned to placebo, 2 of whom discontinued treatment. Overall, 34 adverse events developed during the treatment period in >5% of patients, 18 in 16 patients on sapropterin and 16 in 15 patients on placebo; the nature of the event was in most cases consistent with disease progression (Table I in the online-only Data Supplement). Generally, adverse events were mild to moderate and resolved without the need to discontinue study medication.

Discussion

Vasomotor function seems to be a key pathogenetic mechanism that underlies the phenotypic manifestations of CADASIL, a rare disease for which no specific treatment is currently available. We report here the first randomized, controlled, double-blind trial of treatment targeting endothelial function in patients with confirmed CADASIL. In this phase II multicenter study, supplementation with sapropterin, an analog of BH4, essential cofactor of eNOS in NO synthesis, did not improve the primary end point, endothelium-dependent vasodilation.

The rationale for targeting endothelium-dependent vasodilation in CADASIL rests on the molecular mechanisms of the disease, NOTCH3 accumulation in vascular smooth muscle cells cytoplasmic membrane and within the basement membrane of capillaries between the pericytes and endothelial cells, on previously reported morphological abnormalities in endothelial cells and laboratory alterations of endothelial function in CADASIL patients,[1,23–25,39] and on the association between biomarkers of endothelial function and the clinical phenotype.[40] RH-PAT expresses microvascular function, and its correlation with cerebral blood flow[35] in patients with CADASIL suggests that characterization of peripheral vascular function may represent a convenient, although indirect, marker of brain vascular function, potentially useful to limit the number of patients needed in therapeutic studies addressing the onset and progression of clinical manifestation of CADASIL.

As to minimize the confounding effects of ageing on endothelial function, we limited recruitment to patients <65 years, our population had a mean age of 45.5 years, which corresponds to the reported average at the clinical onset of CADASIL symptoms in the literature.[1] Consistently with this early stage of overt disease, we observed an unsurprisingly low frequency of new cerebrovascular events (3.3/100 person-years) during the study, less than one-third of the average overall incident stroke rate, that supports our choice of a surrogate end point for treatment effect.

As in previous reports,[40] overall 54% of our CADASIL subjects had ≥1 CV risk factor. CV risk factors damage endothelial cells lowering NO bioavailability and, although ED

Table 1.   Baseline Characteristics

	Sapropterin, n=32	Placebo, n=29
Male sex	15 (47%)	18 (62%)
Age, y	46 (8)	45 (9)
Body mass index, kg/m^2	27 (4)	28 (5)
Systolic blood pressure, mm Hg*	118 (10)	124 (15)
Diastolic blood pressure, mm Hg*	77 (10)	80 (10)
Heart rate, bpm	70 (10)	67 (10)
Neurological history		
Migraine	7 (22%)	10 (35%)
Epilepsy	1 (3%)	5 (17%)
Psychiatric disturbances	5 (17%)	6 (22%)
Vision disturbances	…	4 (14)
Gait disturbances	6 (19%)	7 ((24%)
Previous stroke	7 (22%)	9 (31%)
Number of strokes*	2.3 (1.5)	2.4 (1.6)
Previous TIA	5 (16%)	12 (41%)
Number of TIAs*	2.2 (1.8)	2.6 (2.8)
Previous TIA and stroke	12 (37%)	18 (62%)
Years since symptom onset*	7 (9)	7 (7)
Age at symptom onset	37 (12)	37 (10)
Cardiovascular risk factors (≥1)	16 (50%)	17 (59%)
Current smoker	8 (25%)	8 (28%)
Hypertension	3 (10%)	8 (28%)
Hypercholesterolemia	8 (25%)	9 (31%)
Diabetes mellitus	…	2 (7%)
Biochemistry		
Total cholesterol (mmol/L)*	5.22 (0.83)	4.96 (0.90)
HDL-cholesterol (mmol/L)*	1.40 (0.34)	1.34 (0.41)
LDL-cholesterol (mmol/L)*	3.20 (0.80)	3.00 (0.93)
Triglycerides (mmol/L)*	1.29 (0.67)	1.17 (0.53)
Fasting glucose (mmol/L)*	4.55 (0.66)	4.93 (0.77)
Creatinine (mmol/L)*	71.3 (13.2)	73.9 (17.6)
Drug treatment		
Antiplatelet agents	23 (72%)	23 (79%)
Statins	4 (12%)	8 (28%)
Calcium channel blockers	7 (22%)	4 (14%)
RAS inhibitors	6 (19%)	6 (21%)
Any drug active on endothelial function†	13 (41%)	11 (38%)
Antiepilepsy agents	2 (6%)	6 (21%)
Antidepressants	3 (9%)	3 (10%)
Neurological status and disability scales		
mNIHSS >1	4 (12%)	4 (14%)
Barthel index ≤80	1 (3%)	2 (7%)
mRs >1 (disabled)	4 (12%)	4 (14%)

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; mNIHSS, modified National Institute of Health Stroke Scale; mRS, modified Rankin scale; RAS, renin–angiotensin-system; and TIA, transient ischemic attack.

*Non-normal distribution. †composite of calcium channel blocker, RAS-inhibitor or statin.

Table 2.   Primary Efficacy End Point in the Intention-to-Treat and Per Protocol Populations

	Baseline	End of Study	Between-Group Differences vs Placebo (95% CI)	P Value
Intention-to-treat population			0.19 (−0.18, 0.56)	0.914
Sapropterin, n=32	2.13 (0.63)	2.16 (0.70)		
Placebo, n=29	2.01 (0.56)	1.85 (0.47)		
Per-protocol population			0.25 (−0.19, 0.70)	0.798
Sapropterin, n=29	2.11 (0.65)	2.12 (0.72)		
Placebo, n=21	2.01 (0.60)	1.76 (0.41)		

P values are for Wilcoxon rank sum test to compare changes from baseline. CI indicates confidence interval.

can develop throughout the entire vascular tree, the circulation of the brain may be particularly susceptible to it.[41] CV risk factors can exacerbate disease progression in CADASIL patients: Singhal et al[42] found an association between smoking and age at onset of lacunar infarcts, whereas Adib-Samii et al[43] observed an increased risk of ischemic stroke in hypertensive and smoker CADASIL patients. BP, lipid, and glucose profile were similar in the 2 treatment arms.

After 24 months, no between-group difference in RH-PAT, our primary end point, achieved statistical significance. Furthermore, by multivariable logistic regression, no clinical or treatment variable was a significant predictor of improved RH-PAT at the end of study.

Several hypotheses should be considered to explain these negative findings.

The administered drug dose may have been too low to exert relevant effects on endothelial function. Sapropterin, the BH4 formulation used in this trial, is approved for treatment of hyperphenylaninemia at a recommended dose of 10 mg/kg/day. BH4 exerts vasodilating effects through enhanced NO synthesis and may decrease BP.[44] We chose doses in the range previously used in studies of chronic BH4 supplementation in patients with hypercholesterolemia (400 mg BID[45]) or ischemic heart disease (400–700 mg QD[46]) to achieve daily averages of 5 mg/kg. These doses were deemed safe to avoid excessive BP decrease in CADASIL patients, who frequently

Table 3.   Proportion of Patients With Worsened Neurological Status and Disability Scales

	mNIHSS	Barthel Index	mRS
Intention-to-treat population			
Sapropterin, n=32	3 (10)	3 (9)	7 (22)
Placebo, n=29	4 (14)	2 (7)	4 (14)
P value	0.925	1.000	0.627
Per-protocol population			
Sapropterin, n=29	2 (7)	2 (7)	6 (21)
Placebo, n=21	4 (19)	2 (10)	3 (14)
P value	0.223	1.000	0.716

mNIHSS indicates modified National Institute of Health Stroke Scale; and mRS, modified Rankin scale.

Table 4.   Multivariable Analysis of Factors Associated to Improved RH-PAT at End of Study

RH-PAT	Stable/Worsened	Improved	OR	95% CI
Covariate	n=41 (67%)	n=20 (33%)		
Age at baseline (years)	47 (39–52)	46 (41–48)	0.972	(0.896, 1.053)
Sex				
Male	23 (56)	10 (50)	1 (ref.)	…
Female	18 (44)	10 (50)	1.362	(0.410, 4.527)
Duration of symptoms	3 (0–9)	5 (1–12)	1.014	(0.946, 1.087)
Cardiovascular risk factors				
0	19 (46)	9 (45)	1 (ref.)	…
≥1	22 (54)	11 (55)	1.023	(0.305, 3.437)
Cerebrovascular events				
0	21 (51)	10 (50)	1 (ref.)	…
≥1	20 (49)	10 (50)	1.293	(0.358, 4.674)
Any drug active on endothelial function*				
0	26 (63)	11 (55)	1 (ref.)	…
≥1	15 (37)	9 (45)	1.679	(0.460, 6.120)
Treatment arm				
Placebo	21 (51)	8 (40)	1 (ref.)	…
Sapropterin	20 (49)	12 (60)	1.757	(0.547, 5.649)

Odds ratios (OR) from multivariate logistic regression of improved RH-PAT at end of study and corresponding 95% confidence intervals (CI), including terms for all covariates shown. RH-PAT indicates reactive hyperemia index by peripheral arterial tonometry.

show relative hypotension[47]; reduction of already low average BP levels could contribute to decreased cerebral blood flow, further white matter damage, and potential cognitive deterioration. At this dose, sapropterin was remarkably safe, with no excess adverse events nor unfavorable BP changes. In fact, although BP values of ≥130 mm Hg were found in 38% of our patients at baseline, average levels in our CADASIL patients (121±13 mm Hg) were lower than the means reported in other chronic sapropterin trials in healthy hypercholesterolemic subjects (129.5±8.5 mm Hg)[45] or ischemic heart disease patients before coronary artery bypass grafting (>132 mm Hg).[46] A dose up–titration schedule based on BP response to a 5 mg/kg challenge might have allowed to safely reach 10 mg/kg averages and to evidence the potential benefit of higher doses.

Also, a ceiling effect might have occurred; although one-third of our CADASIL patients showed definite ED, overall baseline RH-PAT values were higher than expected. Actually among patients with ≥1 CV risk factors, average baseline RH-PAT was 1.89±0.45 and post-treatment RH-PAT was significantly higher on sapropterin (2.22±0.82) than on placebo (1.77±0.44; P=0.037). The inclusion of patients with more advanced disease might have led to evidence of benefit.

Lack of efficacy might also be because of aspects of BH4 pharmacokinetics and pharmacodynamic that are currently incompletely appreciated. BH4 is easily oxidized, thermo and photo labile, and poorly diffusible through the cell membrane. Although sapropterin has overcome many of these problems, for example, through the incorporation of ascorbic acid as

Table 5.   Number of Patients With Adverse Events After Treatment Start

	Sapropterin (n=32), n (%)	Placebo (n=29), n (%)	P Value
Patients who reported adverse events			
No	16 (50.0)	15 (51.7)	
Yes	16 (50.0)	14 (48.3)	1.000
1	3 (9.4)	2 (6.9)	
2	6 (18.7)	6 (20.7)	
≥3	7 (21.9)	6 (20.7)	
Patients who reported serious adverse events			
No	30 (93.7)	25 (86.2)	
Yes	2 (6.3)	4 (13.8)	0.410
1	1 (3.1)	3 (10.3)	
2	0	1 (3.5)	
≥3	1 (3.1)	0	
Patients who reported ischemic cerebral infarction			
No	31 (96.9)	28 (96.5)	
Yes	1 (3.1)	1 (3.5)	1.000

excipient to prevent autoxidation, bioavailability with chronic administration might still have been limited. The ultimate intent of oral supplementation is the elevation of BH4 within endothelial cells. However, the determinants of intracellular BH4 concentration are complex[16,37] and include the anabolism, catabolism, and recycling of BH4 by dihydrofolate reductase, the enzyme that regenerates BH4 from BH2 inside cells. Whether dihydrofolate reductase activity is preserved in the endothelium of diseased blood vessels is poorly understood.

Treatment duration, the longest to date reported in published CV studies of oral BH4 supplementation, that ranged from 1 to 8 weeks,[45,46,48] might represent another factor in lack of efficacy. Chronic BH4 administration has been suggested to determine accumulation of BH2 in endothelial cells, with competitive replacement of eNOS-bound BH4, eNOS uncoupling, and diminished BH4 efficacy.[48]

Study Limitations

Our study has certain limitations. Although the trial cohort was relatively large for a rare disease, such as CADASIL, the study population was, as a result of the eligibility criteria, relatively young and too small to investigate clinical progression.[2] However, we planned the study and sample size to assess a surrogate end point, endothelial function, validated in other CV diseases. Long-term assessment of the effect of endothelial function on the incidence and timing of new cerebrovascular events in preclinical or mildly symptomatic patients may be useful to target treatments, but enrollment in prospective clinical trials of large populations with rare diseases, such as CADASIL, remains a major challenge.[3]

We did not evaluate the effect of sapropterin on cognitive function, a main and common source of disability in CADASIL. However, no association of vasoreactivity with cognitive performance has been previously reported.[35]

The sensitivity to treatment effects of PAT has been validated in CV disease but not in CADASIL; however, there is no

intervention for CADASIL against which it could have been validated.

Many patients were taking ≥1 drug known to improve endothelial function. Statins alone have significant beneficial direct effects on BH4 bioavailability through upregulation of guanosine triphosphate cyclohydrolase-1 (the rate-limiting enzyme in BH4 synthesis) expression and activity. Calcium channel blockers or rennin–angiotensin system inhibitors also have vessel wall-protective and antioxidant properties. Although drug distribution was balanced across treatment arms at baseline, after 24 months, more subjects who completed the study were on vasoprotective drugs in the placebo arm (44%) than in the active treatment group (34%). It has been suggested[46] that patients naïve to vasoprotective drugs may have greater eNOS reserve, hence, show greater improvement in endothelium-dependent vasodilation with sapropterin than subjects who already take such therapies. Among our patients who took drugs active on endothelial function, after 24 months, RH-PAT was higher on sapropoterin than on placebo (2.28 [0.99] versus 1.73 [0.42], *P*=0.082, respectively). The proportion of patients with ED at the end of study was double in the placebo arm. This finding suggests that, even in patients on antioxidant drugs, a progressive deterioration of endothelial function occurred on placebo that might be delayed by sapropterin.

The last-observation–carried forward method used to impute missing values for the ITT population may introduce bias; however, the findings were replicated both using a different method to account for missing values and in the PP population.

Clinical Implications

CADASIL has been considered a model of sporadic small vessel disease (SVD), with a common specific pattern of progressive vascular cognitive impairment.[49,50]

SVD is strongly associated with aging, diabetes mellitus, and hypertension, a cumulative risk factor burden that strongly affects endothelial function. The theoretical benefits of improved NO bioavailibility in SVD are supported by the efficacy of NO modulators in secondary stroke prevention trials.[51,52] Tolerability of agents with vasodilating properties in SVD patients is suggested by findings of the SPS3 trial,[53] where achievement of lower target BP was associated with significant reductions in intracerebral hemorrhage, stroke, and few serious side effects. Sapropterin, a NO modulator, was well tolerated at low doses in our CADASIL population with normal–low BP values. Because of the prevalence and effect of SVD, with which CADASIL shares structural microvascular damage, the exploration of the potential benefits of sapropterin over and above current vasoprotective drugs, with appropriate dose-finding designs, may be warranted and safe in SVD patients with borderline or high-normal BP.

Conclusions

In this phase II–randomized, double-blind, placebo-controlled trial, sapropterin, an analog of BH4 that increases NO bioavailability, was not effective on endothelium-dependent vasodilation. Sapropterin was safe and well-tolerated at the average dose of 5 mg/kg/day.

The finding of worsening endothelium-dependent vasodilation over 2 years during placebo treatment warrants further investigation of the relation of endothelial function to disease progression in CADASIL patients.

Appendix

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Sources of Funding

Disclosures

Dr Pantoni is Section editor for Stroke and Editorial Board member for Acta Neurologica Scandinavica and Cerebrovascular Diseases. The other authors disclose no conflict of interest.

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Supplemental Material

Table I Distribution of adverse events affecting >5% of patients

	Sapropterin (n=18)	Placebo (n=16)
Transient Neurologic deficit	6	6
Headache	3	4
Migraine	2	4
Depressive symptoms	3	-
Ischemic cerebral infarction	2	2
Near fainiting	2	-