Nimodipine protects vascular and cognitive function in an animal model of cerebral small vessel disease

Zhiyuan Yang, PhD [a*], Frédéric Lange, PhD [b], Yiqing Xia, MRes [a],
Casey Chertavian, MSc [a], Katerina Cabolis, PhD [a],
Marija Sajic, PhD [a], David J. Werring, FRCP PhD [c], Ilias Tachtsidis, PhD [b] and
Kenneth J. Smith, PhD [a*]

[a] Department of Neuroinflammation, UCL Queen Square Institute of Neurology, University College London, London, UK; [b] Department of Medical Physics and Biomedical Engineering, University College London, London, UK; [c] Stroke Research Centre, Department of Brain Repair and Rehabilitation, UCL Queen Square Institute of Neurology, University College London, London, UK.

*Co-corresponding authors

Dr. Zhiyuan Yang
Email: zhiyuan.yang.19@ucl.ac.uk
Twitter: @HelenZYang

Prof. Kenneth Smith
Email: k.smith@ucl.ac.uk
Department of Neuroinflammation, UCL Queen Square Institute of Neurology, 1 Wakefield Street, London, WC1N 1PJ

Running title: Therapeutic value of nimodipine in a model of cSVD

Word count:
5703 words (not including Supplemental Material)

Abbreviations:
bNIRS: broadband near infrared spectroscopy; CCB: calcium channel blocking agent; cSVD; cerebral small vessel disease; CVR: cerebrovascular reactivity; HbDiff: haemoglobin difference (i.e., difference between oxyhaemoglobin and deoxyhaemoglobin, $HbDiff=HbO_2$-HHb); HbO_2: oxyhaemoglobin; HbT: haemoglobin total (i.e., total haemoglobin, $HbT=HbO_2+HHb$); HHb: deoxyhaemoglobin; NMC: neurometabolic coupling; NORT: novel object recognition test; NVC: neurovascular coupling; oxCCO: oxidized cytochrome-c oxidase; SHRSP: spontaneously hypertensive stroke-prone rat: VCID: vascular cognitive impairment and dementia; VSMCs: vascular smooth muscle cells

Abstract:

Background: Cerebral small vessel disease (cSVD) is a common cause of vascular cognitive impairment and dementia (VCID). There is an urgent need for preventative treatments for VCID, and reducing vascular dysfunction may provide a therapeutic route. Here we investigate whether the chronic administration of nimodipine, a CNS-selective dihydropyridine calcium channel blocking agent, protects vascular, metabolic and cognitive function in an animal model of cSVD, the spontaneously hypertensive stroke-prone rat (SHRSP).

Methods: Male SHRSPs were randomly allocated to receive either a placebo (n=24) or nimodipine (n=24) diet between 3 and 6 months of age. Animals were examined daily for any neurological deficits, and vascular function was assessed in terms of neurovascular and neurometabolic coupling (NVC and NMC) at 3 and 6 months of age, and cerebrovascular reactivity (CVR) at 6 months of age. Cognitive function was evaluated using the novel object recognition test (NORT) at 6 months of age.

Results: Six untreated control animals were terminated prematurely due to strokes, and one due to seizure, but no treated animals experienced strokes and so had a higher survival (p=0.0088). Vascular function was significantly impaired with disease progression, but nimodipine treatment partially preserved NVC and NMC, indicated by larger (p<0.001) and more prompt responses (p<0.01), and less 'habituation' upon repeated stimulation (p<0.01). Also, animals treated with nimodipine showed greater CVR, indicated by larger dilation of arterioles (p=0.015) and increase in blood flow velocity (p=0.001). This protection of vascular and metabolic function achieved by nimodipine treatment was associated with better cognitive function (p<0.001) in the treated animals.

Conclusions: Chronic treatment with nimodipine protects from strokes, and from vascular and cognitive deficits in SHRSP. Nimodipine may provide an effective preventive treatment for stroke and cognitive decline in cSVD.

Key words: Spontaneously hypertensive stroke-prone rat; dihydropyridine calcium blocking agent; neurovascular coupling; cerebrovascular reactivity; novel object recognition test; near infrared spectroscopy.

Introduction

Cerebral small vessel disease (cSVD) describes disorders that affect the arterioles, capillaries and venules of the brain[1], which account for about 1 in 3 ischaemic strokes, 80% of strokes due to intracerebral haemorrhage[2], and contribute to up to 45%of all cases of dementia[3]. The mechanisms by which vascular pathology develops to vascular cognitive impairment and dementia (VCID) remain uncertain, impeding development of effective treatments. Indeed, current management of cSVD, which focuses on controlling risk factors (such as hypertension), show inconsistent outcomes in cognitive protection and limited efficacy [4,5]. Early treatment is likely to be most beneficial, raising the need for biomarkers predictive of later cognitive decline.

The vascular dysfunction associated with cSVD has emerged as a promising biomarker of forthcoming cognitive decline. Apart from cerebral hypoperfusion[6], there are important deficits in *dynamic* vascular function, namely neurovascular coupling (NVC)[e.g.7] and cerebrovascular reactivity (CVR)[e.g.8] , mainly due to increased arterial stiffness and endothelial dysfunction[9]. In fact, it is the deficit in dynamic function of the vasculature, rather than in cerebral perfusion at resting state, that best predicts clinical outcomes[10], including poor cognitive performance[11]. Vascular dysfunction impairs cognition, probably through mechanisms including hypoxic neuronal damage[12], amyloid deposition (as in the 'two-hit' hypothesis of Alzheimer's disease)[13], and disruption of functional network connectivity evaluated with MRI[14]. However, despite many observations on vascular dysfunction and on cognitive decline in patients with cSVD, little attention has been focused on protecting vascular physiology to improve cognitive function.

Nimodipine, a CNS-selective dihydropyridine calcium channel blocking agent (CCB), has been widely used in the clinic as a vasodilating agent to reduce vasospasm after subarachnoid haemorrhage. Nimodipine is highly lipophilic compared with nifedipine and other dihydropyridine CCBs[15], and its permeability across the blood-brain barrier increases its accessibility to vascular components outside the endothelial tight junctions, including pericytes and vascular smooth muscle cells (VSMCs). Nimodipine is well tolerated and it has been shown to be beneficial in a range of neurological diseases[16]. The drug is also of some benefit in vascular and other dementias[17], but the mechanisms are uncertain and the clinical benefits are often small.

We have found significant protection by nimodipine in animal models of multiple sclerosis[18] and here we investigate whether chronic treatment with nimodipine protects vascular and cognitive function in the spontaneously hypertensive stroke-prone rat (SHRSP), a widely used animal model of cSVD[19].

Methods

The authors declare that all supporting data are available within the article and its online supplementary files. Male SHRSPs were bred in house and maintained in a 12 hour light/dark cycle, with food and water *ad libitum*. The animals (see **Figure 1**) were randomly allocated at three months of age to either receive a control diet (placebo group), or a diet containing 200mg/kg nimodipine (Ssniff Spezialdiäten GmbH, Germany) (nimodipine group). Dosing from three months was chosen because the pathology of cSVD is usually not fully developed at this time in SHRSPs[20], mimicking a clinical scenario where treatment starts when patients show risk factors of cSVD, but not yet severe pathology. Both diets continued for three months,

during which the animals were daily monitored for any abnormal behaviour or distress that may indicate a stroke. Affected animals were promptly culled (see

Figure 2), and so were excluded from blood pressure measurement, and from study evaluating vascular and cognitive function (next). Observations were made regarding neurovascular coupling (NVC) and neurometabolic coupling (NMC) when the animals were 3 months of age, and at 6 months of age, i.e. after 3 months on diet, using non-invasive broadband near infrared spectroscopy (bNIRS) system[21] developed in-house and illustrated in **Figure 3 a**. The peak response amplitude, peak latency and 'habituation' upon repeated stimulation were quantified with the bNIRS recordings of oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb), haemoglobin difference (HbDiff; i.e. HbO_2-HHb) and haemoglobin total (HbT; i.e. HbO_2+HHb), as well as oxidized cytochrome-c oxidase (oxCCO)[22]. Vascular function was also evaluated by monitoring cerebrovascular reactivity (CVR) upon CO_2 stimulation, in a proportion of animals after 3 months on diet, through an exposed cranial window (**Figure 4 a)**. Arteriolar diameter and blood flow velocity were quantified before and after CO_2 stimulation, and the extent of increase in both parameters was used to indicate CVR. As a measure of cognitive function, the novel object recognition test (NORT)[23] was performed after 3 months on diet (**Figure 5 a**). The NORT was conducted before the aforementioned experiments on vascular function to avoid any effect of anaesthesia or surgery on cognitive performance. The time each animal spent interacting with novel and familiar objects was quantified, from which the difference score (t_{novel}-$t_{familiar}$) and the discrimination ratio ($t_{novel}/(t_{novel}$+$t_{familiar})$) were calculated. All experiments (**Figure 1**) were performed in accordance with the UK Home Office Animals (Scientific Procedures) Act (1986). The animal study protocol follows the ARRIVE 2 guidelines[24], and was approved by the Ethics Committee of University College London and the UK Home Office. *Please refer to the Supplemental Material for detailed methods.*

Results

Chronic treatment of nimodipine reduces incidence of stroke in SHRSP

Animals in both placebo and nimodipine groups exhibited elevated systolic blood pressure (placebo: 226.3±12.9mmHg; nimodipine: 204.9±11.9mmHg) which was greatly above the normal range (around 150mmHg; see[19]). Several animals (n=6) in the placebo group showed signs of seizure, or distress, namely hunching, reduced movement and aggressiveness upon handling, at 17, 24, 26, 28, 29 and 31 weeks of age, and were promptly terminated. Gross examination of the brains at post-mortem revealed ischaemic or haemorrhagic lesions (

Figure 2 a). No animal in the nimodipine group showed any abnormal behaviour when alive, or any visible lesions upon gross examination after termination, resulting in a significantly better outcome for animals in the group treated with nimodipine (

Figure 2 b; p=0.009).

Nimodipine preserves neurovascular and neurometabolic coupling

Vascular function was assessed by monitoring the NVC and NMC in both groups of animals at 3 and 6 months of age (**Figure 3 b, c and d**, and **Table S1**). The highest response amplitude (**Figure 3 b**) and the shortest peak latency (**Figure 3 c**) in NVC (HbO_2, HHb, HbDiff and HbT) and NMC (oxCCO) were observed at 3 months of age. If left untreated, the animals exhibited a progressive decline in NVC and NMC with age, indicated by decreased amplitude and

prolonged latency in the 6-month-old placebo group. This decline was significantly less pronounced in the group treated with nimodipine. Upon repeated stimulation with short intervals, the amplitude of the responses typically decreases (as seen in **Figure 3 a**), which we term as habituation. Habituation was least prominent in 3-month-old group, as indicated by a beta value closest to 0, and it became more prominent with age (**Figure 3 d**). Animals treated with nimodipine were significantly protected from habituation.

Nimodipine preserves cerebrovascular reactivity

CVR upon CO_2 inhalation was assessed as a measure of vascular function in a proportion of the animals from both groups (placebo n=10; nimodipine n=14) at the end of the study (**Figure 4 a**). Dilation in cortical arterioles upon CO_2 stimulation was observed in all animals (**Figure 4 b**), but the percentage increase in arteriolar diameter was significantly larger in the nimodipine group compared with the placebo group (**Figure 4 c**; 24.21±14.63% vs. 43.45±22.76%, p=0.015). The increase in diameter correlated with a greater increase in blood flow velocity measured in the venules (**Figure 4 d**; 16.63±6.27% vs. 29.44±10.14%, p=0.001). As indicated by the trend in linear regressions in **Figure 4 f**, the effect of nimodipine in protecting arteriolar reactivity was more prominent in smaller arterioles. When subgrouping arterioles into those with small diameter (<30μm) vs. large diameter (>=30μm), the protective effect of nimodipine was significant only in the small arterioles (**Figure 4 e** (animal-level comparison; small arterioles: 26.51±15.55% vs. 48.87±20.36%, p=0.005; large arterioles: 23.25±21.68% vs. 39.52±26.20%, p=0.066) **and 4 f** (vessel-level comparison; small arterioles: 25.79±22.62% vs. 52.46±28.34%, p<0.001; large arterioles: 25.60±21.50% vs. 42.03±29.69%, p=0.017)).

The protection in vascular function is associated with a preservation of cognitive function

The changes in discrimination ratio of both the placebo and nimodipine groups across 10 minutes of testing phase is shown in **Figure 5 b-i.** Animals in both groups showed an increase in the discrimination ratio during the first minute of the testing phase (**Figure 5 b-ii**), indicating that the animals were initially attracted to the novel object. However, as the testing phase went on, the discrimination ratio of the placebo group dropped to showing no particular interest, whereas the nimodipine group continued to show a greater interest in the novel object (**Figure 5 b-i**), suggesting that they recognised it as being novel. We chose the first 5 minutes (300 seconds) as a representative time period for quantification of the test phase[23], which showed that the nimodipine group had significantly higher discrimination ratio (**Figure 5 c**; 0.46±0.12 vs. 0.62±0.12; p<0.001) and difference score (**Figure 5 d**; -1.10±2.65sec vs. 3.09±4.39sec; p<0.001) compared with the placebo group. Furthermore, the discrimination ratio and difference score were not different from their reference level (0.5 and 0, respectively) in the placebo group (discrimination ratio: p=0.96; difference score: p=0.08), but were significantly higher than reference levels in the nimodipine group (discrimination ratio: p<0.001; difference score: p=0.002). Cognitive function, as indicated by the difference score, was significantly correlated with vascular function, as represented by the response amplitude of HbT (**Figure 5 e**; p=0.009).

Discussion

The study has investigated the therapeutic value of chronic oral administration of nimodipine in SHRSP, an animal model of cSVD. The results show that the treatment provides protection

from two of the major clinical consequences of cSVD, namely stroke and cognitive deficits[25]. The protection of cognitive function is associated with the preservation of NVC and CVR, suggesting that dynamic vascular function is a therapeutic target for VCID.

Impaired vascular function, represented by decreased NVC and/or CVR, has been extensively reported in animal models of cSVD[26] including SHRSP[27], and in patients with various forms of cSVD[28]. Our findings support these observations by revealing that upon somatosensory stimulation the changes in cerebral blood volume (HbT) and tissue oxygenation (HbO$_2$, HHb and HbDiff) are reduced and delayed as animals advance from three to six months of age. More importantly, using our bNIRS system we have been able not only to detect the reduction in vascular function, but also to show a parallel reduction in *mitochondrial* function, as revealed by measurement of oxCCO. It is reasonable to predict that the lack of adequate mitochondrial function will result in impaired neuronal function, and it follows that the reduced vascular and mitochondrial function provides a sufficient explanation for the cognitive decline observed in both animal models and patients.

The findings show that NVC and NMC, together with CVR, can be partly protected by chronic treatment with nimodipine. We recognise that anaesthesia, including isoflurane anaesthesia, can influence the physiological properties of the vasculature, but the same anaesthesia was employed for both experimental and control groups, reducing the impact of any such effects. The effects of nimodipine on vascular function have received very little attention, but our results are consistent with the available observations showing preservation of NVC by nimodipine in spreading depolarisation[29,30]. In both of these studies, acute administration of nimodipine, either directly into the parenchyma through a cranial window or via intraperitoneal injection, showed a protective effect on the dynamic vascular function. It is encouraging that our findings show the feasibility of achieving chronic protection simply by oral administration of the drug.

The preservation of dynamic vascular function was found to be associated with protection of cognitive function in the same batch of SHRSP. The cognitive protection is in agreement with previous studies showing the beneficial effects of acute or sub-acute administration of nimodipine in animal models of ischaemic stroke.g.[31], and by chronic (52 weeks) treatment[32] of patients with vascular dementia. The current findings suggest that the protection of cognitive function is at least partly dependent on the preservation of dynamic vascular function.

We believe the current study is the first to examine the beneficial effects of chronic nimodipine treatment initiated at an early stage of cSVD, revealing a protection from cognitive decline and a preservation of vascular function. Previous studies have shown that sub-acute (three weeks) or chronic (10 to 20 weeks) treatment with nimodipine starting in older SHRSP (e.g. 40 weeks of age) achieves a similar pattern of protection as in our study of younger animals, namely an improvement in survival, and a preservation of cognitive function and microvascular integrity [33-35] However, the mechanism(s) responsible for the protection remain unclear, but it is notable that nimodipine reduces blood pressure, as in our study, and a number of studies have implicated hypertension as a risk factor for pathologies related to cSVD. Indeed, some anti-hypertensive treatments have the beneficial effects of reducing the microanatomical changes in spontaneously hypertensive rats[36], and also reducing cSVD pathologies[37]. Antihypertensives can delay cognitive impairment[37], and reduce the risk of dementia in patients[38,39], but the beneficial effects are not ubiquitous. Thus, a clinical trial managing cardiovascular risks for

over 6 years resulted in a significantly reduced incidence of hypertension, but not of all-cause dementia[40]. Similarly, lowering blood pressure over 11 years in young patients with recent lacunar stroke also did not protect cognitive function[41]. Furthermore, some antihypertensive drugs lower the risk of Alzheimer's disease and related dementias, but others do not[42].

Against this background it is notable that treatment with dihydropyridine calcium channel blocking agents such as nimodipine is widely beneficial. Nimodipine protects against the consequences of sub-cortical vascular dementia[32], and administration 7-14 days after cerebral infarction for 3 months results in memory improvement[43], perhaps partly achieved by protection from spreading depolarisation and consequent spreading ischaemia[16]. Indeed, use of dihydropyridine calcium channel blocking agents improves cognitive performance independently of blood pressure level, suggesting a specific neuroprotective effect of this pharmacological class[44]. It is therefore noteworthy that apart from reducing blood pressure, nimodipine is directly protective to neurons[16], and anti-inflammatory effects in reducing astrocyte and microglial activation[16], with protective effects on pericytes and oligodendrocytes[16,45]. Even *in vitro*, where effects on blood flow are not involved, nimodipine still shows cell type-independent suppression of stress-dependent apoptosis[46], and protection of tissue slices[47] and the neurovascular unit[48]. In summary, it is likely that the mechanisms underlying the neuroprotective effects of nimodipine are multifactorial.

Interestingly, it appears that nimodipine has particular benefits that are not necessarily shared by other dihydropyridine calcium channel blocking agents[47]. Thus, the protective effects of nimodipine are not adequately reproduced by nifedipine in slice cultures[49], or by nicardipine in a systematic review[50]. Indeed, although nimodipine is used to improve outcome in subarachnoid haemorrhage on the basis that it avoids delayed vasoconstriction, the actual mechanism of action is now being re-evaluated since other interventions that successfully target angiographic vasospasm have not improved outcome[51]. In fact, we anticipate that the observed protection of vascular and cognitive function by nimodipine is at least partly through a preservation of cellular components of the neurovascular unit, even behind the blood-brain barrier due to the fact that nimodipine is highly lipophilic. Nimodipine improves endothelial function (BBB integrity) after anoxia-reoxygenation in an in vitro study where effects on blood flow are not involved [48]. Indeed, drugs protecting endothelial cells preserves CVR [52] and cognitive function [53] in patients with lacunar stroke, without affecting blood pressure. While the cellular mechanism of the therapeutic effect of nimodipine in the current study still needs investigating, the findings suggest a pathway where vascular dysfunction contributes to cognitive decline, and nimodipine protects vascular function.

Conclusion

The results show that chronic treatment with nimodipine protects from strokes and the decline in both cognition and vascular function that normally occur during prolonged disease in cSVD and its SHRSP model. Further, the findings indicate that the decline in cognitive function is partly due to a decline in dynamic vascular function, and that nimodipine protects dynamic vascular function. Our findings suggest a rationale for further testing of nimodipine in randomised trials in patients with cSVD, and for further validation of dynamic vascular function as an early biomarker not only for VCID, but also potentially for other types of dementia and neurodegenerative diseases.

Acknowledgments

Sources of Funding

Disclosure Statements

DJW has received: speaking honoraria from Bayer; speaking and chairing honoraria from Alexion Pharmaceuticals, Inc. and Novo Nordisk; and consultancy fees from Alnylam Pharmaceuticals, Bayer and Novo Nordisk. Prof. Tachtsidis is the CEO and founder of Metabolight Ltd.

Supplemental Material

ARRIVE guideline checklist

Expanded materials and methods

Table S1

Figure legend

Figure 1. Brief illustration of the experimental design. CVR: cerebrovascular reactivity; NMC: neurometabolic coupling; NORT: novel object recognition test; NVC: neurovascular coupling; SHRSP: spontaneously hypertensive stroke-prone rat.

Figure 2. Some animals in the placebo group experienced a stroke, but no animals in the nimodipine group. **(a)** Some animals in the placebo group, but not in the nimodipine group, showed severe distress, associated with ischaemic (white arrowhead) and/or haemorrhagic (white arrow) stroke lesions in the brain. **(b)** The incidence of 'adverse events' was significantly higher in the placebo group compared with the nimodipine group. Red: nimodipine; n=0/24 adverse outcome. Blue: placebo; n=6/24 adverse outcome. Statistical significance was determined by Chi-square, **p<0.01.

Figure 3. The data show that, overall, NVC and NMC were impaired as the animals aged, but were partially protected by chronic treatment with nimodipine. **(a)** NVC and NMC were assessed by recordings from the cortex showing changes in oxygenation and metabolism upon somatosensory stimulation, using our customized bNIRS system. In the placebo group (grey bars; **b-d**), both NVC (indicated by change in oxyhaemoglobin HbO_2; deoxyhaemoglobin, HHb; total haemoglobin, HbT; and difference in haemoglobin, HbDiff), and NMC (indicated by changes in oxidized cytochrome-c oxidase, oxCCO) showed high response amplitude **(b)**, short peak latency **(c)**, and low vulnerability to habituation upon repeated stimulation **(d)** at 3 months of age, but these measures were significantly impaired at 6 months of age (blue and red bars). However, response amplitude **(b)**, peak latency **(c)**, and vulnerability to habituation **(c)** were all significantly protected in the nimodipine group (red), compared with the placebo group (blue). Grey: animals at 3 months of age; n=18. Red: nimodipine; n=24. Blue: placebo; n=17. Bar plots are indicated as mean ± SD. Statistical significance was determined by independent t-test, ns not significant, *p<0.05, **p<0.01, ***p<0.001.

Figure 4. Animals in the nimodipine group showed better CVR upon CO_2 stimulation. **(a)** CVR was assessed by the dilation of arterioles and increase in venular blood flow velocity upon CO_2 stimulation, as viewed through a cranial window. **(b)** Representative images of cortical vasculature before and after CO_2 stimulation in both groups. The cortical arteriole (white arrows) of an animal in the nimodipine group showed a larger dilation after CO_2 compared with that of an animal in the placebo group, while the diameter of venules in both groups remained unchanged. Scale bar=100μm. **(c)** The percentage increase in arteriolar diameter was significantly higher in the nimodipine group, corresponding with **(d)** a significantly higher percentage increase in venular blood flow velocity, indicating a better CVR response. The protective effect of nimodipine treatment on the reactivity of arterioles upon CO_2 stimulation was more significant in small arterioles with diameter <30μm, indicated by both **(e)** animal-level and **(f)** vessel-level comparisons. Red: nimodipine; n=14. Blue: placebo; n=10. Bar plots are indicated as mean ± SD. Statistical significance was determined by independent t-test, ns not significant, *p<0.05, **p<0.01, ***p<0.001.

Figure 5. Animals treated with nimodipine showed better cognitive function, correlating with better vascular function. **(a)** Cognitive function was evaluated with the NORT, consisting of five consecutive days of handling, a 10-minute training phase and a 10-minute testing phase. **(b-i and b-ii)** The discrimination ratio was plotted for both groups across the 10-minute testing phase, which showed distinct differences between the groups. After 5 minutes of the initiation

of the testing phase, the **(c)** discrimination ratio and **(d)** difference score were both significantly higher in the nimodipine group compared with the placebo group. Furthermore, both parameters were significantly higher than reference levels (i.e. 0.5 for discrimination ratio and 0 for difference score) in the nimodipine group, but not in the placebo group. **(e)** Cognitive function, indicated by the difference score, was significantly correlated with NVC, represented by the response amplitude of HbT. Red: nimodipine; n=24. Blue: placebo; n=18. Bar plots are indicated as mean ± SD; box plots as min to max. Statistical significance was determined by independent t-test and linear regression, ns not significant, $*p<0.05$, $**p<0.01$, $***p<0.001$.

References

1. Horsburgh K, Wardlaw JM, van Agtmael T, Allan SM, Ashford MLJ, Bath PM, Brown R, Berwick J, Cader MZ, Carare RO, et al. Small vessels, dementia and chronic diseases - molecular mechanisms and pathophysiology. *Clin Sci (Lond)*. 2018;132:851-868. doi: 10.1042/CS20171620

2. Debette S, Schilling S, Duperron MG, Larsson SC, Markus HS. Clinical Significance of Magnetic Resonance Imaging Markers of Vascular Brain Injury: A Systematic Review and Meta-analysis. *JAMA Neurol*. 2019;76:81-94. doi: 10.1001/jamaneurol.2018.3122

3. Hachinski V, Einhaupl K, Ganten D, Alladi S, Brayne C, Stephan BCM, Sweeney MD, Zlokovic B, Iturria-Medina Y, Iadecola C, et al. Preventing dementia by preventing stroke: The Berlin Manifesto. *Alzheimers Dement*. 2019;15:961-984. doi: 10.1016/j.jalz.2019.06.001

4. Novak V, Hajjar I. The relationship between blood pressure and cognitive function. *Nat Rev Cardiol*. 2010;7:686-698. doi: 10.1038/nrcardio.2010.161

5. Iadecola C, Duering M, Hachinski V, Joutel A, Pendlebury ST, Schneider JA, Dichgans M. Vascular Cognitive Impairment and Dementia: JACC Scientific Expert Panel. *J Am Coll Cardiol*. 2019;73:3326-3344. doi: 10.1016/j.jacc.2019.04.034

6. Ferro DA, Mutsaerts HJ, Hilal S, Kuijf HJ, Petersen ET, Petr J, van Veluw SJ, Venketasubramanian N, Yeow TB, Biessels GJ, Chen C. Cortical microinfarcts in memory clinic patients are associated with reduced cerebral perfusion. *J Cereb Blood Flow Metab*. 2020;40:1869-1878. doi: 10.1177/0271678X19877403

7. Switzer AR, Cheema I, McCreary CR, Zwiers A, Charlton A, Alvarez-Veronesi A, Sekhon R, Zerna C, Stafford RB, Frayne R, et al. Cerebrovascular reactivity in cerebral amyloid angiopathy, Alzheimer disease, and mild cognitive impairment. *Neurology*. 2020;95:E1333-E1340. doi: 10.1212/wnl.0000000000010201

8. Sleight E, Stringer MS, Clancy U, Arteaga C, Jaime Garcia D, Hewins W, Jochems ACC, Hamilton OKL, Manning C, Morgan AG, et al. Cerebrovascular Reactivity in Patients With Small Vessel Disease: A Cross-Sectional Study. *Stroke*. 2023;54:2776-2784. doi: 10.1161/strokeaha.123.042656

9. Webb AJS, Werring DJ. New Insights Into Cerebrovascular Pathophysiology and Hypertension. *Stroke*. 2022;53:1054-1064. doi: 10.1161/STROKEAHA.121.035850

10. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. *Lancet Neurol*. 2019;18:684-696. doi: 10.1016/S1474-4422(19)30079-1

11. Rane S, Koh N, Boord P, Madhyastha T, Askren MK, Jayadev S, Cholerton B, Larson E, Grabowski TJ. Quantitative cerebrovascular pathology in a community-based cohort of older adults. *Neurobiol Aging*. 2018;65:77-85. doi: 10.1016/j.neurobiolaging.2018.01.006

12. Davies AL, Desai RA, Bloomfield PS, McIntosh PR, Chapple KJ, Linington C, Fairless R, Diem R, Kasti M, Murphy MP, Smith KJ. Neurological deficits caused by tissue hypoxia in neuroinflammatory disease. *Ann Neurol*. 2013;74:815-825. doi: 10.1002/ana.24006

13. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci*. 2011;12:723-738. doi: 10.1038/nrn3114

14. Ter Telgte A, van Leijsen EMC, Wiegertjes K, Klijn CJM, Tuladhar AM, de Leeuw FE. Cerebral small vessel disease: from a focal to a global perspective. *Nat Rev Neurol*. 2018;14:387-398. doi: 10.1038/s41582-018-0014-y

15. Tomassoni D, Lanari A, Silvestrelli G, Traini E, Amenta F. Nimodipine and its use in cerebrovascular disease: evidence from recent preclinical and controlled clinical studies. *Clin Exp Hypertens*. 2008;30:744-766. doi: 10.1080/10641960802580232

16. Carlson AP, Hanggi D, Macdonald RL, Shuttleworth CW. Nimodipine Reappraised: An Old Drug With a Future. *Curr Neuropharmacol*. 2020;18:65-82. doi: 10.2174/1570159X17666190927113021

17. Lopez-Arrieta JM, Birks J. Nimodipine for primary degenerative, mixed and vascular dementia. *Cochrane Database Syst Rev*. 2002:CD000147. doi: 10.1002/14651858.CD000147

18. Desai RA, Davies AL, Del Rossi N, Tachrount M, Dyson A, Gustavson B, Kaynezhad P, Mackenzie L, van der Putten MA, McElroy D, et al. Nimodipine Reduces Dysfunction and Demyelination in Models of Multiple Sclerosis. *Ann Neurol*. 2020;88:123-136. doi: 10.1002/ana.25749

19. Kaiser D, Weise G, Moller K, Scheibe J, Posel C, Baasch S, Gawlitza M, Lobsien D, Diederich K, Minnerup J, et al. Spontaneous white matter damage, cognitive decline and neuroinflammation in middle-aged hypertensive rats: an animal model of early-stage cerebral small vessel disease. *Acta Neuropathol Commun*. 2014;2:169. doi: 10.1186/s40478-014-0169-8

20. Hannawi Y, Caceres E, Ewees MG, Powell KA, Bratasz A, Schwab JM, Rink CL, Zweier JL. Characterizing the Neuroimaging and Histopathological Correlates of Cerebral Small Vessel Disease in Spontaneously Hypertensive Stroke-Prone Rats. *Front Neurol*. 2021;12:740298. doi: 10.3389/fneur.2021.740298

21. Kaynezhad P, Mitra S, Bale G, Bauer C, Lingam I, Meehan C, Avdic-Belltheus A, Martinello KA, Bainbridge A, Robertson NJ, Tachtsidis I. Quantification of the severity of hypoxic-ischemic brain injury in a neonatal preclinical model using measurements of cytochrome-c-oxidase from a miniature broadband-near-infrared spectroscopy system. *Neurophotonics*. 2019;6:045009. doi: 10.1117/1.NPh.6.4.045009

22. Bale G, Elwell CE, Tachtsidis I. From Jobsis to the present day: a review of clinical near-infrared spectroscopy measurements of cerebral cytochrome-c-oxidase. *J Biomed Opt*. 2016;21:091307. doi: 10.1117/1.JBO.21.9.091307

23. Bevins RA, Besheer J. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nat Protoc*. 2006;1:1306-1311. doi: 10.1038/nprot.2006.205

24. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *BMC Vet Res*. 2020;16:242. doi: 10.1186/s12917-020-02451-y

25. Ostergaard L, Engedal TS, Moreton F, Hansen MB, Wardlaw JM, Dalkara T, Markus HS, Muir KW. Cerebral small vessel disease: Capillary pathways to stroke and cognitive decline. *J Cereb Blood Flow Metab*. 2016;36:302-325. doi: 10.1177/0271678X15606723

26. van Veluw SJ, Hou SS, Calvo-Rodriguez M, Arbel-Ornath M, Snyder AC, Frosch MP, Greenberg SM, Bacskai BJ. Vasomotion as a Driving Force for Paravascular Clearance in the Awake Mouse Brain. *Neuron*. 2020;105:549-561 e545. doi: 10.1016/j.neuron.2019.10.033

27. Calcinaghi N, Wyss MT, Jolivet R, Singh A, Keller AL, Winnik S, Fritschy JM, Buck A, Matter CM, Weber B. Multimodal imaging in rats reveals impaired neurovascular coupling in sustained hypertension. *Stroke*. 2013;44:1957-1964. doi: 10.1161/STROKEAHA.111.000185

28. Sleight E, Stringer MS, Marshall I, Wardlaw JM, Thrippleton MJ. Cerebrovascular Reactivity Measurement Using Magnetic Resonance Imaging: A Systematic Review. *Front Physiol*. 2021;12. doi: 10.3389/fphys.2021.643468

29.   Szabo I, O MT, Torok Z, Varga DP, Menyhart A, Frank R, Hantosi D, Hunya A, Bari F, Horvath I, et al. The impact of dihydropyridine derivatives on the cerebral blood flow response to somatosensory stimulation and spreading depolarization. *Br J Pharmacol*. 2019;176:1222-1234. doi: 10.1111/bph.14611

30.   Menyhart A, Balint AR, Kozak P, Bari F, Farkas E. Nimodipine accelerates the restoration of functional hyperemia during spreading oligemia. *J Neurochem*. 2023;n/a. doi: 10.1111/jnc.15792

31.   Chen Y, Guo Z, Peng X, Xie W, Chen L, Tan Z. Nimodipine represses AMPK phosphorylation and excessive autophagy after chronic cerebral hypoperfusion in rats. *Brain Res Bull*. 2018;140:88-96. doi: 10.1016/j.brainresbull.2018.03.019

32.   Pantoni L, del Ser T, Soglian AG, Amigoni S, Spadari G, Binelli D, Inzitari D. Efficacy and safety of nimodipine in subcortical vascular dementia: a randomized placebo-controlled trial. *Stroke*. 2005;36:619-624. doi: 10.1161/01.STR.0000155686.73908.3e

33.   Meneses A, Terron JA, Ibarra M, Hong E. Effects of nimodipine on learning in normotensive and spontaneously hypertensive rats. *Behav Brain Res*. 1997;85:121-125. doi: 10.1016/s0166-4328(97)87580-8

34.   Luiten PG, de Jong GI, Schuurman T. Cerebrovascular, neuronal, and behavioral effects of long-term Ca2+ channel blockade in aging normotensive and hypertensive rat strains. *Ann N Y Acad Sci*. 1994;747:431-451. doi: 10.1111/j.1749-6632.1994.tb44427.x

35.   Farkas E, De Jong GI, Apro E, Keuker JIH, Luiten PGM. Calcium antagonists decrease capillary wall damage in aging hypertensive rat brain. *Neurobiol Aging*. 2001;22:299-307. doi: 10.1016/s0197-4580(00)00225-6

36.   Sabbatini M, Tomassoni D, Amenta F. Influence of treatment with Ca2+ antagonists on cerebral vasculature of spontaneously hypertensive rats. *Mech Ageing Dev*. 2001;122:795-809. doi: 10.1016/s0047-6374(01)00233-0

37.   Markus HS, Erik de Leeuw F. Cerebral small vessel disease: Recent advances and future directions. *Int J Stroke*. 2023;18:4-14. doi: 10.1177/17474930221144911

38.   Lennon MJ, Lam BCP, Lipnicki DM, Crawford JD, Peters R, Schutte AE, Brodaty H, Thalamuthu A, Rydberg-Sterner T, Najar J, et al. Use of Antihypertensives, Blood Pressure, and Estimated Risk of Dementia in Late Life: An Individual Participant Data Meta-Analysis. *JAMA Netw Open*. 2023;6:e2333353. doi: 10.1001/jamanetworkopen.2023.33353

39.   Hughes D, Judge C, Murphy R, Loughlin E, Costello M, Whiteley W, Bosch J, O'Donnell MJ, Canavan M. Association of Blood Pressure Lowering With Incident Dementia or Cognitive Impairment: A Systematic Review and Meta-analysis. *JAMA*. 2020;323:1934-1944. doi: 10.1001/jama.2020.4249

40.   Moll van Charante EP, Richard E, Eurelings LS, van Dalen J-W, Ligthart SA, van Bussel EF, Hoevenaar-Blom MP, Vermeulen M, van Gool WA. Effectiveness of a 6-year multidomain vascular care intervention to prevent dementia (preDIVA): a cluster-randomised controlled trial. *The Lancet*. 2016;388:797-805. doi: 10.1016/s0140-6736(16)30950-3

41.   Pearce LA, McClure LA, Anderson DC, Jacova C, Sharma M, Hart RG, Benavente OR, Investigators SPS. Effects of long-term blood pressure lowering and dual antiplatelet treatment on cognitive function in patients with recent lacunar stroke: a secondary analysis from the SPS3 randomised trial. *Lancet Neurol*. 2014;13:1177-1185. doi: 10.1016/S1474-4422(14)70224-8

42.   Marcum ZA, Gabriel N, Bress AP, Hernandez I. Association of New Use of Antihypertensives That Stimulate vs Inhibit Type 2 and 4 Angiotensin II Receptors With Dementia Among Medicare Beneficiaries. *JAMA Netw Open*. 2023;6:e2249370. doi: 10.1001/jamanetworkopen.2022.49370

43.   Sze KH, Sim TC, Wong E, Cheng S, Woo J. Effect of nimodipine on memory after cerebral infarction. *Acta Neurol Scand*. 1998;97:386-392. doi: 10.1111/j.1600-0404.1998.tb05971.x

44.   Watfa G, Rossignol P, Kearney-Schwartz A, Fay R, Bracard S, Felblinger J, Boivin JM, Lacolley P, Zannad F, Benetos A. Use of calcium channel blockers is associated with better cognitive performance in older hypertensive patients with subjective memory complaints. *J Hypertens*. 2010;28:2485-2493. doi: 10.1097/HJH.0b013e32833e4108

45.   Boltz F, Enders M, Feigenspan A, Kirchner P, Ekici A, Kuerten S. Nimodipine Exerts Beneficial Effects on the Rat Oligodendrocyte Cell Line OLN-93. *Brain Sci*. 2022;12:476. doi: 10.3390/brainsci12040476

46.   Leisz S, Simmermacher S, Prell J, Strauss C, Scheller C. Nimodipine-Dependent Protection of Schwann Cells, Astrocytes and Neuronal Cells from Osmotic, Oxidative and Heat Stress Is Associated with the Activation of AKT and CREB. *Int J Mol Sci*. 2019;20. doi: 10.3390/ijms20184578

47.   Hohmann U, Ghadban C, Hohmann T, Kleine J, Schmidt M, Scheller C, Strauss C, Dehghani F. Nimodipine Exerts Time-Dependent Neuroprotective Effect after Excitotoxical Damage in Organotypic Slice Cultures. *Int J Mol Sci*. 2022;23. doi: 10.3390/ijms23063331

48.   Xue Q, Liu Y, Qi H, Ma Q, Xu L, Chen W, Chen G, Xu X. A novel brain neurovascular unit model with neurons, astrocytes and microvascular endothelial cells of rat. *Int J Biol Sci*. 2013;9:174-189. doi: 10.7150/ijbs.5115

49.   Lecht S, Rotfeld E, Arien-Zakay H, Tabakman R, Matzner H, Yaka R, Lelkes PI, Lazarovici P. Neuroprotective effects of nimodipine and nifedipine in the NGF-differentiated PC12 cells exposed to oxygen-glucose deprivation or trophic withdrawal. *Int J Dev Neurosci*. 2012;30:465-469. doi: 10.1016/j.ijdevneu.2012.05.007

50.   Feigin VL, Rinkel GJ, Algra A, Vermeulen M, van Gijn J. Calcium antagonists in patients with aneurysmal subarachnoid hemorrhage: a systematic review. *Neurology*. 1998;50:876-883. doi: 10.1212/wnl.50.4.876

51.   Al-Tamimi YZ, Orsi NM, Quinn AC, Homer-Vanniasinkam S, Ross SA. A review of delayed ischemic neurologic deficit following aneurysmal subarachnoid hemorrhage: historical overview, current treatment, and pathophysiology. *World Neurosurg*. 2010;73:654-667. doi: 10.1016/j.wneu.2010.02.005

52.   Blair GW, Janssen E, Stringer MS, Thrippleton MJ, Chappell F, Shi Y, Hamilton I, Flaherty K, Appleton JP, Doubal FN, et al. Effects of Cilostazol and Isosorbide Mononitrate on Cerebral Hemodynamics in the LACI-1 Randomized Controlled Trial. *Stroke*. 2022;53:29-33. doi: 10.1161/STROKEAHA.121.034866

53.   Wardlaw JM, Woodhouse LJ, Mhlanga, II, Oatey K, Heye AK, Bamford J, Cvoro V, Doubal FN, England T, Hassan A, et al. Isosorbide Mononitrate and Cilostazol Treatment in Patients With Symptomatic Cerebral Small Vessel Disease: The Lacunar Intervention Trial-2 (LACI-2) Randomized Clinical Trial. *JAMA Neurol*. 2023. doi: 10.1001/jamaneurol.2023.1526