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Rodent Models of Vascular Cognitive Impairment

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Abstract

Vascular cognitive impairment dementia (VCID), which is an increasingly important cause of dementia in the elderly, lacks effective treatments. Many different types of vascular disease are included under the diagnosis of VCID, including large vessel disease with multiple strokes, and small vessel disease with lacunar infarcts and white matter disease. Animal models have been developed to study the multiple forms of VCID. Because of its progressive course, small vessel disease (SVD) is thought to be the optimal form of VCID for treatment. One theory is that the pathophysiology involves hypoxic hypoperfusion resulting in injury to the white matter and neuronal death. Bilateral occlusion of the common carotid arteries (BCAO) in a normotensive rat, which reduces cerebral blood flow, induces hypoxia with white matter damage; this model has been used to test drugs to block the injury. Another model is the spontaneously hypertensive/stroke prone rat (SHR/SP). Hypertension leads to small vessel disease resulting in progressive damage to the white matter, cortex and hippocampus. Bilateral carotid artery stenosis (BCAS) with coils or ameroid constrictors produces a slower development of changes than BCAO, avoiding the acute ischemia. A few studies have been done with the two-clip, two-vessel occlusion renal model for induction of hypertension. There are benefits and drawbacks to each of these models with the model selected depending on the type of vascular damage that is to be studied. This review describes the most commonly used models, and the drugs that have been used to reduce the damage.

Keywords

Vascular cognitive impairment; neuroinflammation; spontaneously hypertensive rats; matrix metalloproteinases

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INTRODUCTION

Vascular disease has been identified as an increasingly important cause of dementia, making it imperative that treatments be developed for the growing number of patients with dementia related to vascular disease (Gorelick et al., 2011; Snyder et al., 2015). Vascular cognitive impairment dementia (VCID) is the term suggested for all forms of dementia related to vascular diseases (Corriveau et al., 2016). The term includes multiple forms of stroke, which can be separated into large vessel and small vessel disease (SVD). Multiple pathophysiologies result in a heterogeneous patient population that greatly complicates treatment trials, and makes it necessary to stratify patients into subgroups for clinical trials to be possible with reasonably sized patient groups.

Treatment trials are possible when the course of the disease in the untreated state is known. The small vessel form of VCID has a progressive course as opposed to the random course of the large vessel forms. Most efforts to model VCID have used SVD as the optimal form for future trials. The hallmark of SVD is lacunar strokes and extensive injury to the white matter. A major impediment to the use of animal models is the heterogeneity of the causes of vascular dementia, including embolic and thrombotic disease, hypertension and other risk factors, hereditary causes, and hypoxic hypoperfusion. While each of these causes can be simulated in an animal, those with progressive rather than sporadic changes are optimal for testing therapies.

Several animal models have emerged as the most useful to study vascular causes of dementia: 1) hypoxic hypoperfusion secondary to bilateral carotid artery occlusion (BCAO) in normotensive rats, 2) small vessel disease (SVD) secondary to hypertension in the spontaneously hypertensive/stroke prone rat (SHR/SP), 3) rat and mouse models of bilateral carotid artery stenosis (BCAS), and 4) mouse models of global ischemia (Guerrini et al., 2002; Nishio et al., 2010; Wakita et al., 1995; Walker and Rosenberg, 2010). All of these models produce damage to the deep white matter with behavioral changes. Hypoperfused white matter develops lesions, making the BCAO model clinically relevant, but it lacks the long-term vascular changes produced by hypertension, the most common vascular risk factor, which is present in a large proportion of the patients with vascular cognitive impairment (VCI) that have extensive changes in the white matter. This review will focus on the animal models most relevant for SVD, including BCAO, SHR/SP, renal hypertension, and gradual carotid stenosis. The pros and cons of each model will be described along with the drugs that have been tested in these models.

Pathophysiology of Hypertensive Brain Injury

Hypoxia and inflammation are the main factors involved in the damage to the deep white matter. Cerebral vasculature in both the rat and human brains is supplied from the surface, penetrating into the deep white matter, which is the most vulnerable region for hypoxia due to the watershed effect (De Reuck et al., 1980). As a result of long-standing hypertension, the cerebral blood vessels have narrowed lumens with distension of the outer walls from fibrosis (Rigsby et al., 2007).

We have demonstrated hypoxic conditions in the white matter of SHR/SP with electron paramagnetic resonance (EPR), which allows for minimally invasive measurements of the level of oxygen in the white matter over an extended period of time by implanting an oxygen-sensing crystal in the tissues (Weaver et al., 2014). EPR showed that the oxygen content in the white matter begins to gradually increase by the ninth week of life in the SHR/SP, reaching a maximum at twelve weeks after birth. Following unilateral carotid artery occlusion (UCAO) and abnormal diet, which are started at 12 weeks, the oxygen content in the white matter falls precipitously to hypoxic levels where it remains until the animal dies around week 16. The elevation of the oxygen level may represent an attempt by the brain cells to increase the oxygen extraction to overcome the onset of hypoxia, which is referred to as “misery-perfusion” because the cerebral blood flow falls and the oxygen extraction rises (Derdeyn et al., 2002).

As a consequence of the reduction in brain oxygen levels a series of molecular events are initiated, culminating in an inflammatory response. Central to the hypoxia-induced inflammation is the induction of hypoxia inducible factor-1 α (HIF-1 α) (Semenza, 2014). HIF-1 α induces a large number of genes involved in both injury and repair. Hypoxia induces the *Furin* gene, which encodes a convertase involved in a number of important reactions, including the formation of the protein, Furin, which is involved in the activation of proteases, such as the matrix metalloproteinases (Cunningham et al., 2005). Induction of MMPs opens the blood-brain barrier (BBB) with vasogenic edema damaging myelinated fibers of the deep white matter. The MMPs disrupt the BBB by attacking basal lamina and tight junction proteins (Yang et al., 2007).

Matrix metalloproteinases are a family of 26 enzymes some of which are constitutively expressed and others are induced (Rosenberg, 2016). Normally, gelatinase A (MMP-2) is present in the brain in the astrocytic endfeet close to the cerebral vessels; the latent 72-kDa MMP-2 is activated to the 62-kDa form by the action of a trimolecular complex formed by membrane-bound MMP (MMP-14) and tissue inhibitor to metalloproteinases-2 (TIMP-2). When the three molecules join together, which occurs close to the endothelial cell due to the tethering action of MMP-14, the activated MMP-2 loosened the molecules in the basal lamina surrounding the endothelial cells and the tight junction proteins. As the hypoxic injury proceeds there is release of cytokines, which induce the action of the more damaging inducible MMPs, namely MMP-3 and MMP-9 which further damage the cerebral blood vessels (Candelario-Jalil et al., 2009).

Activation of the MMPs in the hypoxic brain creates conditions for collateral damage to other tissues. MMPs attack myelinated fibers breaking them down into myelin basic protein (Chandler et al., 1995). The attack by enzymes on myelin was first demonstrated with the enzyme plasmin secreted from stimulated macrophages. Since this was a nonimmunological process, it was referred to “by-stander” demyelination (Cammer et al., 1978).

Kidney injury precedes the onset of brain lesions in the SHR/SP with dietary manipulation. Low levels of proteinuria (40mg/day) appear at the time of initial blood vessel injury in the SHR/SP brains; this includes vasogenic edema, lacunar infarcts and focal cell loss. With the worsening of the proteinuria to over 100mg/day changes are seen in the brain in the

subventricular zone with higher levels of nestin, GFAP, and doublecortin, indicating the emergence of immature neural stem cells and that only new astrocytes can survive. At this stage, no MRI-detectable lesions are seen (Cova et al., 2013).

The consequences of the narrowed, stiffened blood vessels is a reduction in cerebral blood flow along with an inability to respond quickly to the changing needs for blood flow (Pillai and Mikulis, 2015). If the hypoxia is severe, cytokines are activated leading to inflammation through the action of the inducible MMPs, MMP-3 and MMP-9, which have activator protein-one (AP-1) and nuclear factor- κ B (NF- κ B) transcription sites in their genes. The basal lamina and the tight junction proteins are disrupted, resulting in the opening of the BBB with vasogenic edema spreading through the loose fiber construction of the white matter. The anti-inflammatory, MMP inhibitor, Minocycline, attenuates the damage to the white matter (Cai et al., 2008).

MRI shows the increased water content as a white signal on T2-weighted images. The area of the T2-weighted hyperintensities is markedly increased in animals with chronic hypertension. Apparent diffusion coefficient (ADC) increases represent the increased permeability of vessels, representing vasogenic edema (Newcombe et al., 2013). Fractional anisotropy (FA) decreases reflect the demyelination and/or axonal damage (Budde et al., 2011). Arterial spin labeling (ASL), which measures cerebral blood flow, is reduced.

Interaction of hypertensive vascular disease and Alzheimer's disease—Elderly patients with dementia often show multiple pathological changes, including amyloid plaques, phosphorylated Tau, α -synuclein, and microinfarcts (Sonnen et al., 2011). Aging and hypertension result in a high incidence of patients with several types of pathologies rather than pure vascular disease or Alzheimer's disease, making mixed dementia the most common form in the elderly patient population with dementia (Schneider et al., 2007; Snyder et al., 2015). There is increased interest in mechanisms of brain injury when multiple types of pathology are present. The interaction of amyloid with vascular disease leads to acceleration of the brain damage. Hypertension can lead to accumulation of amyloid and phosphorylated Tau (Bueche et al., 2014; Schreiber et al., 2012). Cortical microinfarcts (CMI) observed in brains of patients with Alzheimer's disease tend to be co-localized with cerebral amyloid angiopathy (CAA) (Greenberg et al., 2014). In the CAA mouse model with chronic cerebral hypoperfusion due to BCAS induced with microcoils, there was accelerated deposition in the leptomeninges of amyloid beta ($A\beta$), and some developed microinfarcts; CAA mice without hypoperfusion exhibited very few leptomeningeal $A\beta$ depositions and no microinfarcts by 32 weeks of age, suggesting that cerebral hypoperfusion accelerates CAA, and promotes CMI (Okamoto et al., 2012).

To examine the link between midlife hypertension and the onset of AD later in life, one research group chemically induced chronic hypertension in the transgenic Swedish-Dutch-Iowa mutation (TgSwDI (mouse model of AD in early adulthood. Hypertension accelerated cognitive deficits in the Barnes maze test, microvascular deposition of $A\beta$, vascular inflammation, BBB leakage, and pericyte loss; in addition, hypertension induced hippocampal neurodegeneration at an early age in this mouse line, establishing this as a

useful research model of AD with mixed vascular and amyloid pathologies (Kruger et al., 2015).

Long-term studies in the BCAS model showed deficits in working memory due to frontal-subcortical circuit damage. Impaired working memory was seen at 5 to 6 months, and hippocampal atrophy at 8 months with neuronal loss (Nishio et al., 2010; Shibata et al., 2007).

Animal Models

Bilateral Carotid Artery Occlusion in Normotensive Rats and Mice—Bilateral carotid artery occlusion (BCAO) was developed as a model for hypoxic hypoperfusion, which leads to secondary white matter damage (Wakita et al., 1994; Wakita et al., 1995; Yamada et al., 2011b). It has been used in many studies of the pathophysiology of white matter damage and to test the effect of various therapies (Wakita et al., 1995). The tissue is assumed to be hypoxic although direct measurements of tissue oxygen levels have not been made. Occlusion of both carotids results in a temporary severe reduction in cerebral blood flow, which gradually returns to pre-occlusion values over weeks to months (Farkas et al., 2007).

In the BCAO model, astrocytes react to the oligemia (Kitamura et al., 2012). The age of the animal influences the extent of astrocytosis with the older animals showing more changes (Wakita et al., 1994). There is enlargement of the basilar artery to compensate for the restricted cerebral blood flow, which may explain the return to normal blood flow over time. One week after BCAO the optic nerve shows astrocytosis, which eventually leads to severe shrinkage of the optic tract; the damage to the visual system interferes with the cognitive tests (Schmidt-Kastner et al., 2005). Following BCAO there is demyelination related to both the hypoxia and the breakdown of the BBB. Horseradish peroxidase (HRP) studies using light and electron microscopy show leakage of the HRP into the paramedian part of the corpus callosum, which can be seen by 3 hours and persists for 3 days before BBB integrity is restored. Electron microscopy shows proliferation of collagen fibrils in the thickened basal lamina (Ueno et al., 2002). Matrix metalloproteinases (MMPs) are expressed in the hypoxic tissue and contribute to the opening of the BBB and myelin damage (Kitamura et al., 2012; Nakaji et al., 2006). In the chronic cerebral hypoperfusion model there is induction of MMP-2 by 3 days, but not of MMP-9 (Ihara et al., 2001).

We used magnetic resonance imaging (MRI) to demonstrate that 3 days after BCAO in the rat there is disruption of the BBB, which appears as an increase in apparent diffusion coefficients, suggesting vasogenic edema (Sood et al., 2009). Cognitive behavioral tests, including the Morris water maze task, odor discrimination task, and novel object test, show diverse cognitive impairments at 4–5 weeks. These diverse cognitive impairments were accompanied by disintegration of white matter by neuroinflammation, loss of oligodendrocytes, attenuation of myelin density, and disintegration of white matter tracts, demonstrated by histology and MRI diffusion tensor imaging measurements (Jalal et al., 2012).

Chronic oligemia secondary to BCAA has been suggested as a model for Alzheimer's disease with the changes in amyloid and Tau proteins attributed to hypoxia (de la Torre, 2004). While this could explain the secondary formation of pathological changes in amyloid and Tau once the tissue is oligemic, initiating events in the normotensive younger patients appear to be related to neuronal dysfunction.

Since histopathological findings of the ischemic brain of spontaneously hypertensive rats (SHRs) subjected to BCAA are quite similar to those seen in clinical cases, SHR-BCAA model has been used to study the clinical pathophysiologies that occur during hemorrhagic shock, or systemic hypotension. SHRs are more susceptible to cerebral ischemia than normotensive rats. Causes of this increased susceptibility in SHRs compared to normotensive rats are thought to result from several causes, including, smaller diameter of the posterior communicating artery and the basilar artery, thicker vascular wall of the cerebral and carotid arteries, and higher cerebrovascular resistance during maximum vasodilation. Therefore, when subjected to BCAA, SHRs show a severe decrease in cerebral blood flow, the formation of brain edema, and death (Ohtani et al., 2000).

Mice have been used to study the effects of transient carotid artery occlusion; permanent bilateral carotid artery occlusion in the mouse is lethal. In one study of the effect of 17 min transient cerebral ischemia in mice there was an increase in MMP-2, but also an increase in MMP-9 in neurons, which was not seen in the chronically hypoperfused rats, suggesting there may be species differences (Magnoni et al., 2004).

Another model developed for use in the mouse is to constrict both carotid arteries with a tiny coil, producing bilateral carotid artery stenosis (BCAS) (Shibata et al., 2004). The optimal inner diameter of the coil is 0.18-mm. BCAS causes a similar reduction in cerebral blood flow as in the rat BCAA model that also is restored over time as in the BCAA model in the rat. From a clinical perspective, this model mimics patients with stenosis of both carotid arteries. These mouse models have the unique advantage that they can be used in genetically manipulated animals.

A drawback of the BCAA model is that the changes in the cerebral blood flow (CBF) occur acutely and resolve chronically. To overcome this limitation, gradual occlusion of the carotids with a device that constricts over time has been used. Gradual narrowing of both common carotid arteries with the ameroid constrictor device instead of ligation circumvents the acute phase of CBF reduction and acute inflammatory response observed in the BCAA model (Kitamura et al., 2012). Gradual constriction of the carotids results in pathological changes similar to those produced by BCAA in the white matter, but does not damage the gray matter. This is an interesting model that has not been used in drug testing, and its utility for those studies will need to be determined. A recent report described adaptation of the ameroid constrictor to mice that have subcortical infarcts with dementia, which should prove a useful model for use in genetically modified animals (Hattori et al., 2015).

Renovascular Hypertensive Rat Model—Induction of kidney disease effectively raises blood pressure with changes in the brain similar to those seen in patients with white matter disease. The two kidney, two-clip model avoids complicating genetic factors, which may be

affecting the lesions in the genetically modified SHR/SP animals. The induction of hypertension secondary to kidney disease results in damage to the myelin especially in corpus callosum and external capsule, disruption of the BBB, vascular changes on small vessels, and behavioral changes. All of these features are also present in the SHR/SP. A recent study using this model showed that peroxisome proliferator-activated receptor- γ agonist, pioglitazone, reduced the damage to the white matter probably through its action as an anti-inflammatory agent (Lan et al., 2015). Pioglitazone significantly attenuated WM damage in corpus callosum, external capsule and internal capsule. Cognitive impairment in the renovascular model was ameliorated by pioglitazone, which also attenuated arteriolar remodeling and reduced sICAM-1 level in serum. Pioglitazone decreased the proliferation of microglia and astrocytes and lowered the expression of proinflammatory cytokines IL-1 β and TNF- α in the white matter.

Spontaneously hypertensive/stroke prone rats—Hypertension is the major cause of SVD that leads to lacunar strokes and white matter hyperintensities (WMHs) in patients with VCI (Iadecola, 2013). SHR/SP are a genetic variant of the Wistar-Kyoto rats (WKY) that was developed in Japan by Yamori and colleagues; they develop severe hypertension 3 months after birth with systolic pressures generally about 200 mm Hg (Yamori, 1991). On average the SHR/SP live for 9 months when they develop cerebral infarction and hemorrhage. Cerebral blood flow is reduced in the frontal regions by 5 months, which is thought to produce hypoxic conditions. Cerebral infarcts are similar to those found in humans with hypertension (Yamori et al., 1976). Besides strokes the SHR/SP have damage in the white matter that is similar to that seen on MRI in patients with VCID. Dietary manipulation has a major effect on the lifespan: adding salt and reducing protein shortens life by damaging the kidneys and brain at an earlier age (Guerrini et al., 2002). When the high salt, low protein diet is used to accelerate the hypertensive damage, the kidney is severely damaged, which can be followed by the measurement of albumin in the urine (Gianella et al., 2007). Gender is another important factor in the model since females show less involvement than males and live longer (Ballerio et al., 2007).

SHR/SP have a progressive injury to the deep white matter that resembles that seen in humans with the subcortical ischemic vascular disease (SIVD) type of VCID (Rosenberg et al., 2016). Without dietary manipulation, the SHR/SP begin to show changes in the white matter around 4 to 5 months and the infarcts and hemorrhages occur at 9 months. It is possible to shorten the lifespan to 4 months by feeding the animals a high salt, low protein diet referred to as the Japanese Permissive Diet (JPD) (Sironi et al., 2004). The benefit of using dietary manipulation is that the experimental time is shortened, facilitating the gathering of data. The downside is that there is extensive damage to the kidneys and brain with the altered diet, making comparison with the human condition more problematic. As these rats age they develop cerebral infarcts with a tendency to hemorrhage, which leads to death.

The vasculature of the SHR/SP is abnormal, making it more vulnerable to stroke than the WKY (Yamori et al., 1976). Depending on the purpose of the study, various modifications can be made. We have further modified the SHR/SP animal model by using both the JPD and occlusion of one carotid (UCAO) (Jalal et al., 2012). For example, in studies involving

intracerebral hemorrhage, the high salt or JPD are introduced at age 6 weeks, which is four weeks prior to the onset of severe hypertension; this results in hemorrhages around 6 to 8 weeks later. In order to focus on the white matter damage, we have delayed the dietary changes until the 12th week of life, when the hypertension is fully developed. By delaying the abnormal diet, it is possible to study the changes in the white matter and to reduce the incidence of stroke, providing a model that emphasizes the damage to the white matter.

Treatments in Animal Models

The optimal model to use for testing of drugs will vary with the pathological process suspected; a large number of potential treatments have been tested in various animal models (TABLE)., SHR/SP best mimics the subcortical lesions in the white matter seen in elderly patients with hypertensive vascular disease. In normotensive rats, BCAA can be used as a model to study inflammatory mechanisms. For hereditary forms of small vessel disease, mouse models with specific gene manipulations will be needed. Treatments in rats with hypoxic hypoperfusion due to either BCAA or spontaneous hypertension have indicated a number of potential therapeutic agents (Farkas et al., 2007; Pedder et al., 2014). Cyclosporine A suppressed both glial activation and white matter changes after chronic cerebral hypoperfusion, suggesting that immunologic reaction may play a role in the pathogenesis of the white matter changes (Nakaji et al., 2006; Wakita et al., 1995). Cyclosporine A inhibits BBB breakdown in APOE4 transgenic mice by inhibiting cyclophilin A-MMP-9 pathway in pericytes leading to reversal of neuronal dysfunction and secondary neurodegenerative changes (Bell et al., 2012). A number of anti-inflammatory agents and free radical scavenging agents have shown benefit (Farkas et al., 2007). Treatments with minocycline significantly attenuated the hypoxia-ischemia-induced brain injury and improved behavioral performance; the protection of minocycline was associated with its ability to reduce microglial activation (Cai et al., 2008). In the SHR/SP with the JPD and UCAO, treatment with minocycline blocked the full development of white matter injuries seen on MRI in vehicle treated animals and improved behavior in the Morris water maze (Jalal et al., 2015).

Limitations of the various models

In this review, our focus has been on the rodent models that lead to changes in the white matter due to hypoxic hypoperfusion. The hypoxia can be induced by occlusion of the carotids, narrowing of the carotids with coils or ameroid constrictors, stenosis of arteries feeding the kidneys, or with genetically modified rats or mice. The models that have been most extensively studied are the BCAA, BCAS, and SHR/SP. The pathophysiology and ability to test drugs has been shown in each of the models.

While no current model mimics all of the pathological changes seen in VCID, there are benefits and drawbacks of each model. The pros of the BCAA and BCAS models are that they can be done in WKY, which are less expensive than the SHR/SP, they do not have genetic factors, and the time course to produce an injury is short. The cons of the models include the rapid induction of hypoxia by restricting blood flow, which gradually returns to normal, the lack of studies on the actual oxygen levels in the tissue, and the damage to the optic tracts, which are fed by branches of the occluded arteries.

The ischemia produces at the onset of occlusion of the arteries has been overcome with the use of models of carotid stenosis induced by tiny wire coils or ameroid constrictors. These models lack the chronic changes induced in the blood vessels by hypertension, diabetes and other vascular risk factors, which are important in the production of neuroinflammation, which is a finding in patients with the SIVD form of VCID. The ameroid constrictors are an interesting approach that appears to answer some of the concerns created by the permanent occlusion. However, few studies have been done with this model. The effects on the visual system remain to be established.

The genetic manipulations of the WKY that result in chronic hypertension and strokes in the SHR/SP provide a model that more closely mimics the disease in humans with long-standing hypertension, which is the most common vascular risk factor in patients. The lesions in the white matter develop slowly over many months and after about 9 months these animals develop ischemic and hemorrhagic strokes and die. Adding salt to the diet and reducing the protein content of the food accelerates the damage, but also injures the kidneys. These manipulations result in death four weeks after starting the diet, which is best done at 12 weeks of age to induce maximum damage to the white matter before major strokes have occurred. This model can be easily studied with MRI methods, which are very sensitive. Combining MRI with behavioral tests provides a non-invasive way to test drugs and study survival.

Since there are drawbacks to all of the available models, the choice of the optimal one for a given study needs to take into account the major objectives of the study.

CONCLUSIONS

Several models can be used to study the white matter in rodents. Animals differ in species, age, genetic factors, presence or absence of vascular risk factors. Bilateral carotid occlusion in normotensive young or old rats most closely mimics patients with bilateral carotid stenosis, which is an uncommon clinical presentation; these rats have hypoxia hypoperfusion of the white matter, making this a reasonable model for VCI. Hypertensive rats are more closely aligned with the human disease, and they can be either renovascular hypertensive with renal artery clipping or SHR/SP. Since many of the patients have hypertension, the SHR/SP animal model is the most relevant in spite of the confounding genetic effects. Animal models are ideal for determining the pathophysiology and for testing drugs.

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Table 1

Models of Vascular dementia on mice and rats

	Models	Drug Mechanisms	Drug tested	Reference
Renovascular occlusion	RHRSP	TZD	pioglitazone	(Lan et al., 2015).
Chronic Hypertension	SHR	AchEI and MAOI	galantamine(AchEI)+a-glyceryl phosphonyl-choline	(Tayebati et al., 2009).
		Antihypertension	nicardipine	(Amenta and Tomassoni, 2004)
	UCAO-JPD (SHR/SP)	Antiinflammation	minocycline	(Jalal et al., 2015).
Chronic cerebral hypoperfusion	BCAO/BCAS	AchEI and MAOI	donepezil; ASS234 (AchEI); ASS188 (monoamine oxidase); bifemelane hydrochloride	(Kondo et al., 1996; Kwon et al., 2014; Stasiak et al., 2014)
		Antiepileptogenesis	Lamotrigine	(Ozkul et al., 2014)
		Antihypertension	diazoxide+DMSO; Angiotensin 1–7; Ramipril; Perindopril [#] ; aliskiren (a renin inhibitor) [*] ; Mexonidine (11-imidazoline R) [*] ; Clonidine [*]	(Dong et al., 2011; Farkas et al., 2004; Gupta and Sharma, 2014; Kim et al., 2008; Xie et al., 2014; Yamada et al., 2011a)
		Antiinflammation	quercetin; resveratrol; E-selectin; minocycline; Baicalein; AG3340 (MMP-2 inhibitor); SB202190 (p38 MAPK inhibitor)	(Wakita et al., 1995)
		Antiplatelet agents	cilostazol; budilast	(Choi et al., 2016)
		Immunosuppressants	Cyclosporine A; FK506	(Wakita et al., 1995)
		Others	L-carnitine	(Ueno et al., 2015)

This table includes mice and rat models of vascular dementia (VaD), with the mechanism of renovascular hypertension, chronic hypertension and chronic cerebral hypoperfusion.

[#] tested on the model of BCAA on SHR

^{*} tested on the model of mice

We excluded the drugs containing 2 or more ingredients and in which the mechanism are unclear.

Abbreviations; renovascular hypertensive rat-stroke prone (RHRSP), spontaneously hypertensive rat (SHR), unilateral carotid artery occlusion-Japanese permissive diet (UCAO-JPD), bilateral carotid artery occlusion (BCAO), bilateral common carotid artery occlusion (BCAO), bilateral carotid artery stenosis (BCAS), Sprague-Dawley rat (SD rat), spontaneously hypertensive rat-stroke prone (SHR/SP), thiazolidinedione (TZD), monoamine oxidase inhibitor (MAOI), dimethyl sulfoxide (DMSO), angiotensin converting enzyme inhibitor (ACE inhibitor), AchEI (Acetylcholine receptor inhibitor)