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The natural flavonoid pinocembrin: molecular targets and potential therapeutic applications

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Abstract

Pinocembrin is a natural flavonoid compound extracted from honey, propolis, ginger roots, wild marjoram, and other plants. In preclinical studies, it has shown anti-inflammatory and neuroprotective effects as well as the ability to reduce reactive oxygen species, protect the blood-brain barrier, modulate mitochondrial function, and regulate apoptosis. Considering these pharmaceutical characteristics, pinocembrin has potential as a drug to treat ischemic stroke and other clinical conditions. In this review, we summarize its pharmacologic characteristics and discuss its mechanisms of action and potential therapeutic applications.

Keywords

neuroinflammation; neuroprotection; pinocembrin; stroke

Introduction

Pinocembrin (5,7-dihydroxyflavanone, Figure 1) is a natural flavonoid compound that has been identified in honey, propolis, and several plants, such as ginger roots and wild marjoram [1–6]. Apart from natural extraction, pinocembrin has been successfully biosynthesized [7–14] and chemosynthesized [15]. In pharmacological studies, it has shown a variety of properties that could hold promise for treating diseases such as endotoxin shock, cancer, and cardiovascular diseases [16]. It is interesting to note that after oral administration, this compound is well metabolized and absorbed [17–19]. *In vitro*, it has been shown to pass through the blood-brain barrier (BBB) in a passive transport process, which is partly conducted by p-glycoprotein [20]. This finding indicates that pinocembrin might be useful for treatment of diseases in the central nervous system (CNS). In fact, previous studies have shown that pinocembrin has anti-inflammatory and neuroprotective effects and the ability to reduce reactive oxygen species (ROS), protect the BBB, modulate mitochondrial function, and regulate apoptosis. As discussed in detail below, *in vitro* and *in vivo* studies have provided evidence that pinocembrin can protect the brain against damage

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Compliance with ethical standards

The authors declare no conflict of interest.

from ischemic stroke. Therefore, in 2008, pinocembrin was approved by the State Food and Drug Administration of China for clinical trials in patients with ischemic stroke [21], and is currently enrolling patients for Phase II clinical trials [22].

In this review, we summarize recent preclinical studies of pinocembrin and focus on its effects in CNS diseases and related indications. We discuss information regarding the potential targets of pinocembrin, its possible mechanisms of action, existing problems, and potential therapeutic applications.

Pharmacokinetics of pinocembrin

In preclinical studies, two methods have been used to detect pinocembrin in rat plasma. In the first, investigators administered 22.5 mg/kg or 67.5 mg/kg pinocembrin to rats via intravenous injection (i.v.) and then used reversed-phase high-performance liquid chromatography with ultraviolet detection [23]. In the second study, rats were injected i.v. with 10 mg/kg pinocembrin. Then high-performance liquid chromatographic–electrospray ionization–mass spectrometry was used to detect S-pinocembrin and R-pinocembrin in plasma [17]. In clinical trials, high-performance liquid chromatography–mass spectrometry-mass spectrometry has been used to measure pinocembrin in the plasma of healthy volunteers [21] (Table 1).

Biosynthesis and synthesis of pinocembrin analogs

Pinocembrin was first isolated from *Eriodictyon californicum* in 1992 [24]. In plants, pinocembrin is synthesized from phenylalanine by the action of three enzymes: phenylalanine ammonia lyase, 4-coumarate: CoA ligase, and chalcone synthase [25]. Based on this finding, large amounts of pinocembrin have been biosynthesized from recombinant *Escherichia coli* [26,9] and *Saccharomyces cerevisiae* [27] containing an artificial gene cluster. Recently, Kim et al. [28] successfully synthesized pinocembrin from glucose using engineered *E. coli*. To achieve industrial production of pinocembrin, Yuan et al. [15] developed a convenient method to synthesize (+/−)-pinocembrin by using a chiral primary amine or a chiral sulfinamide as resolving agent.

Potential therapeutic applications and mechanism of action

Pinocembrin has been studied in stroke, Alzheimer’s disease, cardiovascular diseases, and atherosclerosis *in vitro* and *in vivo* (Table 2). Its potential application for cancer treatment is not covered by this review.

Stroke

Stroke is the fourth leading cause of death in the United States and one of the leading causes of adult disability. It can also have tremendous economic and social impacts [29]. Many survivors are left with lifelong, devastating neurologic deficits. In the past decade, various studies have demonstrated that pinocembrin can protect against cerebral ischemic injury with a wide therapeutic time window [30]. It has shown neuroprotective [31,32], anti-oxidative [33], and anti-inflammatory effects [5] both *in vitro* and *in vivo*. Pinocembrin

exerts potent protective effects in rats with ischemic stroke [30–33]. Such findings indicate that it is a promising compound for the development of novel, multiple-action drug therapies.

Effects of pinocembrin in animal models of middle cerebral artery occlusion (MCAO)

The first stroke model used to evaluate the efficacy of pinocembrin was MCAO. In rats with permanent cerebral ischemia (pMCAO), Gao et al. [34] showed that pinocembrin (10 mg/kg, i.v.) could reduce brain swelling; improve behavioral deficits; and alleviate neuronal apoptosis, edema of astrocytic end-feet, and the deformation of endothelial cells and capillaries. Moreover, pinocembrin also protected the BBB by decreasing matrix metalloproteinase-9 protein expression and reducing mRNA level of the tight junction protein zonula occludens-1. Acting on the neurovascular unit, pinocembrin reduces the level of proinflammatory cytokines [tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β)], chemokines [intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)], inducible nitric oxide (NO) synthase (iNOS), and aquaporin-4 [5], suggesting that pinocembrin has an anti-inflammatory effect. In a recent study, Wang et al. [35] co-administered pinocembrin and antagonists of epoxyeicosatrienoic acids (EETs) to pMCAO rats. Results showed that pinocembrin suppressed soluble epoxide hydrolase (sEH) activity and that the EET antagonist weakened the beneficial effects of pinocembrin in pMCAO rats. These results indicate that sEH and the EETs might be targets of pinocembrin. sEH metabolizes EETs into less-active dihydroxyeicosatrienoic acids (DHETs) [36–38]. Because of multiple protective properties [39,40], increasing the level of EETs would be beneficial for injured brain. In mouse and human brain, sEH is expressed in multiple brain cell types, such as neurons, astrocytes, and endothelial cells [39,41]. sEH deletion or inhibition has shown protective effects in both ischemic stroke models [42–45] and neurons subjected to oxygen-glucose deprivation [44,46].

Pinocembrin (10 mg/kg) was also shown to improve ischemic outcomes in the MCAO-reperfusion animal model [33]. It was suggested that pinocembrin reduces endoplasmic reticulum (ER) stress and apoptosis by decreasing C/EBP homologous protein (CHOP)/GADD153 and caspase-12 expression via the PERK-elF2α-ATF4 signaling pathway in MCAO rats [47].

Effects of pinocembrin on 4-vessel occlusion (4-VO) animal model

In a 4-VO rat model, 5 mg/kg pinocembrin protected the BBB by regulating the pathological changes of ultrastructure in microvessels, neurons, and glial cells [48]. In addition, Shi et al. [30] further reported that pinocembrin had an antioxidant effect in 4-VO rats. Pinocembrin reduces the compensatory increase of superoxide dismutase activity, malondialdehyde content, and myeloperoxidase activity; modulates the concentration of excitatory and inhibitory amino acids; and lessens the neuronal loss in rats after global cerebral 4-VO ischemia in a dose-dependent manner (1, 5, 10 mg/kg). In terms of the therapeutic time window, it is notable that pinocembrin was still protective even if administered up to 6 hours after 4-VO induction. Thus, it has a wider application window than tissue plasminogen activator (tPA, 3 h) [49], the only approved drug for ischemic stroke.

In vitro studies

Pinocembrin has been shown to increase neuronal viability, decrease lactate dehydrogenase release, inhibit the production of NO and ROS, increase glutathione levels, and downregulate the expression of neuronal NO synthase (nNOS) and iNOS in primary cortical neurons subjected to oxygen–glucose deprivation/reoxygenation (OGD/R) [33]. These results indicate that pinocembrin is neuroprotective *in vitro*.

Pinocembrin is also able to regulate mitochondrial function and apoptosis. In primary neurons subjected to OGD/R, pinocembrin decreased caspase-3 expression and increased PARP degradation [33]. The decrease in caspase-3 activity has also been demonstrated in tunicamycin-induced SH-Sy5y cells [31]. Chinese propolis, which contains pinocembrin, protects against neuronal toxicity induced by ER stress [31]. In the glutamate-induced SH-Sy5y cell line, it has been shown that pinocembrin decreases the release of cytochrome c from mitochondria into cytoplasm and reduces the synthesis of pro-apoptotic Bax.

Pinocembrin also protects the BBB *in vitro*. Meng et al. [48] found that pinocembrin protected cultured rat cerebral microvascular endothelial cells from OGD/R damage and increased mitochondrial membrane potential. These results suggest that pinocembrin exerts not only neuroprotection but also vascular protection, further supporting its therapeutic application in stroke.

Taken together, increasing evidence from *in vivo* and *in vitro* studies supports the idea that pinocembrin could be a candidate drug to treat ischemic stroke. Although the neuroprotective effects of pinocembrin have not been compared directly with other herbal products, acute toxicity testing showed that the LD_{50} of i.v. pinocembrin in mice is greater than 700 mg/kg (unpublished work by Drs. Song Wu and Guanhua Du), suggesting that pinocembrin is an exceptionally safe drug candidate. In addition, pinocembrin may have a wider therapeutic window (up to 6 hours) than the current 3-hour therapeutic window of tPA.

Neurodegenerative diseases

Investigators have also explored the effect of pinocembrin on Alzheimer's disease *in vitro* and *in vivo*. Liu et al. [50] used three types of cells for *in vitro* studies: cells transfected with the receptor for advanced glycation end products (RAGE); an SH-Sy5y cell line that overexpresses amyloid precursor protein with the Swedish mutation (APPsw); and neurons induced with amyloid-b peptide $(A\beta)_{25\text{-}35}$. They demonstrated that pinocembrin (10 μM) inhibits RAGE and its downstream signaling pathways, indicating that the RAGE protein might be a target of pinocembrin, and that pinocembrin also regulates mitochondrion-mediated apoptosis in the APPsw SH-Sy5y cell line. Liu et al. [51] further showed that, in APP/PS1 transgenic mice, oral administration of pinocembrin significantly reduces learning and memory deficits, mainly by inhibiting glial activation and downregulating RAGE-induced p38 mitogen-activated protein kinase (MAPK) expression in APP/PS1 transgenic mice. These data provide strong evidence that pinocembrin might protect the brain by targeting neuroinflammation. Finally, pinocembrin was reported to decrease neurotoxicity and inhibit mitochondrial dysfunction in 1-methyl-4-phenylpyridinium (MPP(+))–treated

SH-Sy5y cells [52] and to attenuate neuronal death through the Nrf2/ARE pathway in 6-OHDA-treated SH-Sy5y cells [53], suggesting a potential application in Parkinson's disease.

Cardiovascular diseases and atherosclerosis

Pinocembrin is thought to affect cardiovascular diseases based on its ability to regulate ApoE and reduce rho kinase (ROCK). Li et al. [54] demonstrated that pinocembrin inhibits the angiotensin II-induced increase in phosphorylation of MYPT1/Thr696 and ROCK1. The inhibition of vascular contraction caused by pinocembrin is mediated, at least in part, by reduction of MYPT1/Thr696 phosphorylation and ROCK1 expression. Moreover, pinocembrin has been shown to inhibit angiotensin II-induced vasoconstriction by suppressing the increase in $[Ca^{2+}]$ and ERK1/2 activation and blocking angiotensin II type I receptor (AT1R) in the rat aorta [54]. Sang et al. [55] showed that combined treatment with simvastatin and pinocembrin for 14 weeks significantly decreased serum lipid levels, improved endothelial function, and reduced atherosclerosis symptoms in $ApoE^{-/-}$ mice. The effect of the combination therapy was better than that with simvastatin alone. These findings suggest that pinocembrin might be used to treat atherosclerosis when combined with other drugs. Yang et al. [56] reported that pinocembrin improved the biological functions of bone marrow-derived endothelial progenitor cells (EPCs) via the PI3K/AKT/eNOS pathway. However, more evidence is needed to confirm the effects of pinocembrin on cardiovascular diseases, including atherosclerosis, both *in vitro* and *in vivo*.

Inflammatory and infectious diseases

As mentioned above, anti-inflammation is one of the main mechanisms of pinocembrin. Therefore, many experiments have explored possible applications of pinocembrin in the treatment of inflammatory diseases. In an *in vitro* study, Soromou et al. [57] demonstrated that pinocembrin inhibits proinflammatory cytokines in the murine macrophage and endotoxin-induced acute lung injury model, partly by decreasing the levels of MAPK and NF-κB activation. *In vivo*, pretreatment with pinocembrin (intraperitoneal, 50 mg/kg) attenuated inflammation and reduced lung injury in a murine model of lipopolysaccharide (LPS)-induced inflammation. Soromou et al. [58] also found that pretreatment with pinocembrin (intraperitoneal, 20 mg/kg or 50 mg/kg) reduced mortality from LPS-induced endotoxin shock in mice; however, posttreatment with pinocembrin failed to exert any therapeutic effects.

Pinocembrin was first discovered to have anti-fungal properties in 1977 [59]. In the decades since, studies have shown that pinocembrin can significantly inhibit the activity of *Penicillium italicum* [60], *Candida albicans* [61–65], *Staphylococcus aureus* [66,67,61,68,69,65,11], *E. coli* [70,61], *Bacillus subtilis*, *Trichophyton mentagrophytes*, *Streptococcus mutans* [71,65,19], and *Neisseria gonorrhoeae* [72]. Recently, Soromou et al. [66] reported that pinocembrin reduced α-haemolysin production and attenuated α-haemolysin-mediated cell injury at low concentrations and protected mice from *S. aureus*-induced pneumonia.

Together, these findings provide evidence regarding pinocembrin's anti-inflammatory mechanisms and highlight its anti-infectious effect, suggesting that pinocembrin could be a drug candidate for treating post-stroke infections.

Discussion and prospect analysis

As a natural compound with good pharmacological and pharmaceutical properties, pinocembrin has wide applications, including ischemic stroke, neurodegenerative diseases, and atherosclerosis (Fig. 2).

As discussed above, pinocembrin has multiple protective properties (Fig.3). **1) In the CNS**, pinocembrin primarily exerts anti-inflammatory activity by inhibiting NF-κB transcription, MAPK signaling pathways, and their downstream gene transcription (pro-inflammatory cytokines). Among these pathways, RAGE might be an important target of pinocembrin to treat neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease. Second, pinocembrin induces antioxidative effects by decreasing superoxide dismutase, malondialdehyde, myeloperoxidase, and ROS. Pinocembrin also inhibits apoptosis by decreasing the synthesis of pro-apoptotic Bax, reducing caspase-3 activity, and reducing ER stress. These actions are mediated by decreasing CHOP/GADD153 and caspase-12 expression via the PERK-elF2α-ATF4 signaling pathway. Third, pinocembrin might inhibit sEH expression/activity in astrocytes that release high levels of EETs to modulate microglial phenotype and function. **2) In the circulatory system**, pinocembrin primarily inhibits vasoconstriction by downregulating the phosphorylation of MYPT and ERK1/2, the expression of ROCK1, and calcium levels. It also improves endothelial function via the PI3K/AKT/eNOS pathway. **3) In other diseases**, pinocembrin exhibits anti-inflammatory and anti-infectious effects and might be used to treat microbial-induced diseases and post-stroke infection.

Although pinocembrin has many potential applications, it is currently being tested for ischemic stroke in Phase II clinical trials. Thus, it is closest to clinical application for this condition. However, many questions remain concerning potential targets and applications of pinocembrin. **1)** Although several signaling pathways have been shown to mediate the effects of pinocembrin, the cellular and molecular targets of pinocembrin in stroke still need further study, in particular, the role of the EET-sEH pathway. **2)** As we reviewed above, one of the most important mechanisms of pinocembrin is anti-inflammation, but the exact effect of pinocembrin on microglial and macrophage function is still unknown. This uncertainty limits the further application of pinocembrin in CNS diseases. **3)** With anti-inflammatory, anti-oxidant, and neuroprotective properties, pinocembrin might have additional applications, such as in hemorrhagic stroke. Inflammation plays an important role in the development of secondary brain injury after hemorrhagic stroke [73]. We suggest that future studies should focus on the cellular and molecular targets of pinocembrin and explore other possible applications for CNS diseases.

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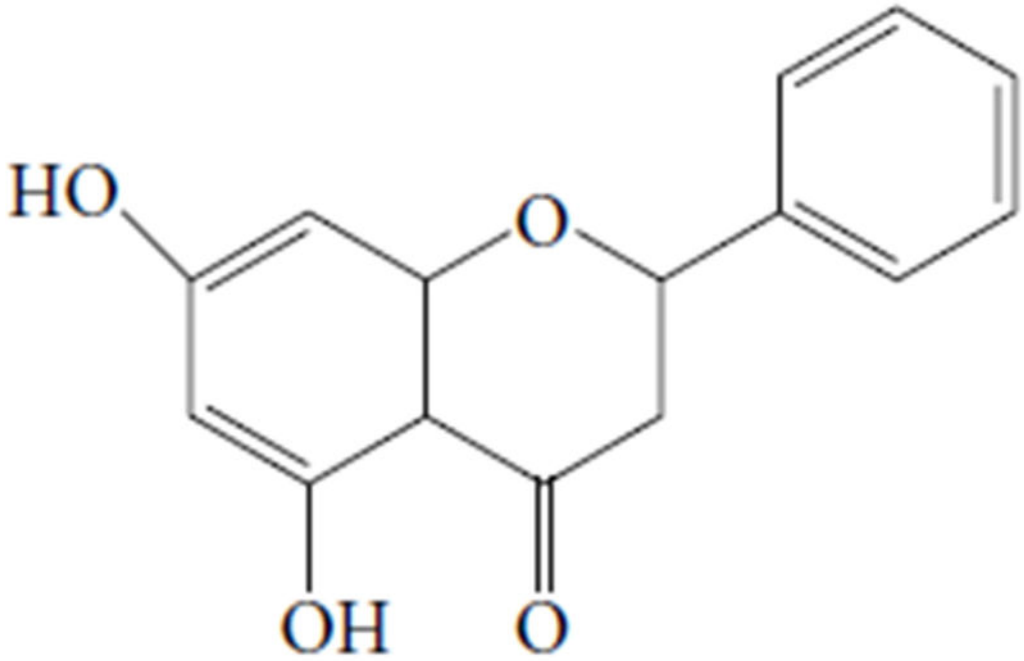

Figure 1.
Chemical structure of pinocembrin.

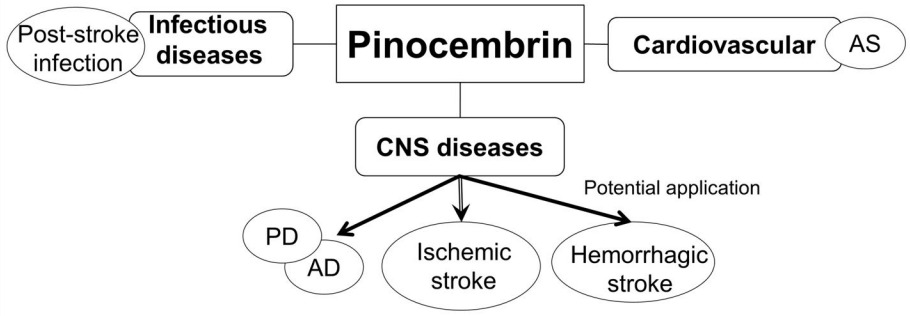

Figure 2.

Potential therapeutic applications of pinocembrin. In the central nervous system (CNS), pinocembrin is a drug candidate for ischemic stroke, but it also exerts protection against Alzheimer's disease (AD) and Parkinson's disease (PD). Pinocembrin has been shown to exert anti-inflammatory and anti-infectious effects (including against bacterial and fungal infections), indicating that it might be useful for treating post-stroke infections. In addition, pinocembrin might have potential application in the treatment of hemorrhagic stroke. In the circulatory system, pinocembrin reduces atherosclerosis (AS) symptoms in animals when combined with simvastatin.

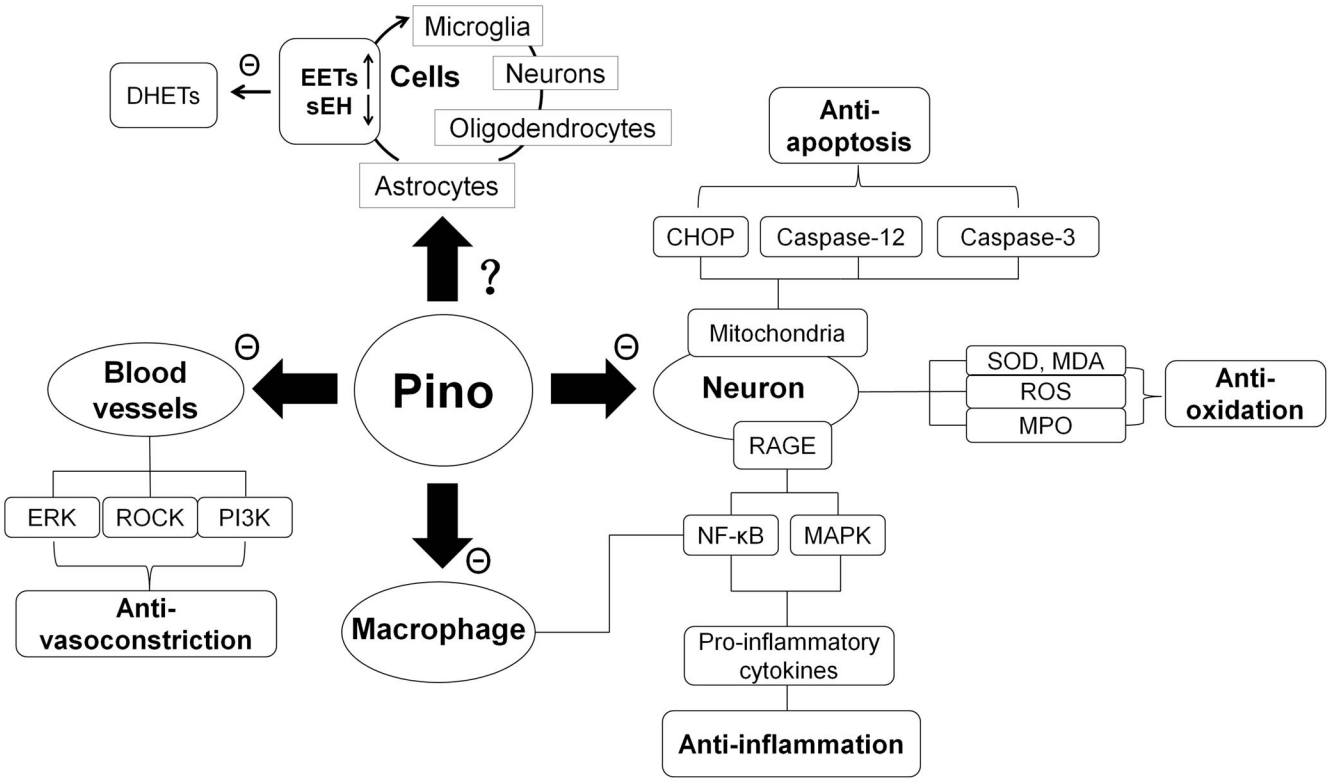

Figure 3.

Main mechanisms of pinocembrin. Clockwise from top: Based on their multiple protective properties, high levels of epoxyeicosatrienoic acids (EETs) released from astrocytes could modulate microglial, neuronal, and oligodendrocyte activity to benefit the injured brain. However, EETs can be metabolized by sEH into less-active dihydroxyeicosatrienoic acids (DHETs). Pinocembrin might inhibit sEH expression/activity and thereby increase EET level in the brain. The effects of pinocembrin on the activity/function of different cell types (neurons, astrocytes, microglia, or oligodendrocytes) and the role of sEH in these cells or cell-cell interactions needs further study. To modulate mitochondrial function, pinocembrin reduces endoplasmic reticulum stress via C/EBP homologous protein (CHOP) and caspase-12, and decreases apoptosis of neurons by suppressing caspase-3 expression/activity. Pinocembrin decreases oxidation, as evidenced by the inhibition of superoxide dismutase (SOD), malondialdehyde (MDA), myeloperoxidase (MPO), and reactive oxygen species (ROS). Pinocembrin decreases expression of the receptor for advanced glycation end products (RAGE) and regulates its downstream targets, including NF-κB and MAPK pathways, to exert an anti-inflammatory effect in macrophages. In the circulatory system, pinocembrin inhibits vasoconstriction via extracellular-signal-related kinase (ERK), Rho-activated kinases (ROCK), and PI3K pathways.

Table 1

Pharmacokinetic characteristics of pinocembrin

Subject	Dose (route)	AUC	T1/2 (min)	CL	Vd
SD rat3[23]	22.5 mg/kg (i.v. injection)	0–2 h: 340.37 ± 2.67 mg/L min	14.61 ± 3.74	0.07 ± 0.02 L/min/kg	
SD rat [23]	67.5 mg/kg (i.v. injection)	0–2 h: 1698.55 ± 335.95 mg/L min	13.93 ± 5.02	0.04 ± 0.01 L/min/kg	
SD rat [17]	10 mg/kg (i.v. injection)	S-Pino: 0–∞ 1.821 ± 0.211 h mg/mL	12.72 ± 8.4	0.092 ± 0.011 L/min/kg	1.758 ± 1.313 L/kg
SD rat [17]	10 mg/kg (i.v. injection)	R-Pino: 0–∞ 1.876 ± 0.427 h mg/mL	13.38 ± 4.98	0.092 ± 0.020 L/min/kg	1.793 ± 0.805 L/kg
Healthy human [21]	20 mg (i.v. drip)	0–8 h: 10,236.0 ± 1547.4 ng/mL min 0–∞: 10,338.1 ± 1539.4 ng/mL min	47.4 ± 14.0	2.0 ± 0.3 L/min	136.6 ± 52.8 L

AUC, area under the curve; CL, clearance; i.v., intravenous; R-Pino, R-Pinocembrin; SD, Sprague-Dawley; S-Pino, S-Pinocembrin; T1/2, half-life; Vd, volume of distribution

Table 2

Main published applications of pinocemrin *in vitro* and *in vivo*

Diseases	Model/organism	Effective dose	Results
Ischemic stroke	pMCAO/rat [34,5]	10 mg/kg (i.v)	+
	MCAO-reperfusion/rat [33,47]	10 mg/kg (i.v)	+
	4-VO/rat [48,30]	5 mg/kg (i.v)	+
	OGD/R-induced primary neurons [33]	10 μM	+
	Glutamate-induced SH-Sy5y [32]	10 μM	+
	OGD/R-induced RCMECs [48]	10 μM	+
Alzheimer's disease	APP/PS1 transgenic mouse [51]	40 mg/kg (i.g.)	+
	APPsw-overexpressing SH-Sy5y cell line [50]	10 μM	+
	$A\beta_{25\text{-}35}$ induced primary neurons [50]	10 μM	+
Atherosclerosis	$ApoE^{-/-}$ mice [56]	20 mg/kg (i.g.)	+
	Ang II-induced rat thoracic aortic rings [54]	100 μM	+
Endotoxin shock	LPS-induced endotoxin shock mice [57]	50 mg/kg (i.p)	+

4-VO, 4-vessel occlusion; Ang II, angiotensin II; i.g., intragastric; i.v., intravascular; LPS, lipopolysaccharide; MCAO, middle cerebral artery occlusion; OGD/R, oxygen–glucose deprivation/reoxygenation; pMCAO, permanent MCAO; RCMECs, rat cerebral microvascular endothelial cells.