Protective effects of cilostazol against hemorrhagic stroke: Current and future perspectives

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ABSTRACT

Cilostazol is a phosphodiesterase-3 inhibitor and is known to have pleiotropic effects including anti-platelet and vasodilatation effects and protective effects on endothelial cells. Cilostazol also reportedly reduced stroke recurrence, poststroke intracranial hemorrhage, and extracranial bleeding in a meta-analysis. Although it is known that cilostazol has the potential to suppress hemorrhagic stroke, the precise mechanisms remained unclear. Therefore, we evaluated the protective effects and mechanisms of cilostazol against hemorrhagic stroke. We found that cilostazol prevented the hemorrhagic transformation induced by focal cerebral ischemia in mice treated with intravenous tissue plasminogen activator or warfarin via protecting endothelial cells and tight junction proteins. We also demonstrated that cilostazol attenuated collagenase-induced intracranial hemorrhage in mice. In vitro studies showed that endothelial cells, pericytes, tight junction proteins, adherence junction proteins, and the basement membrane, which are all components of the blood-brain barrier, were protected by the administration of cilostazol following collagenase injury. These results suggested that cilostazol reduces hemorrhagic stroke by protecting the entire blood-brain barrier. Here, we review the protective effects of cilostazol on the blood-brain barrier that result in the prevention of hemorrhagic stroke, discuss the results we obtained using multiple hemorrhagic stroke models, and introduce potential future applications of cilostazol.

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1. Introduction

Cilostazol is a phosphodiesterase-3 inhibitor that has been approved for use as a vasodilating antiplatelet drug in the treatment of ischemic symptoms in chronic peripheral arterial obstruction or intermittent claudication and for the secondary prevention of cerebral infarction in Asia. Recently, cilostazol was reported to be more effective than aspirin in the secondary prevention of all types of stroke, especially secondary hemorrhagic stroke, in a clinical trial (cilostazol for the prevention of secondary stroke [CSPS 2]) and meta-analysis (1,2). According to these reports, cilostazol has the potential to reduce hemorrhagic stroke.

Currently, only a few treatment strategies for hemorrhagic stroke exist. However, there is no effective treatment for the hemorrhagic transformation (HT) that occurs after the administration of intravenous tissue plasminogen activator (tPA) or anticoagulation therapies like warfarin. Moreover, early decreases in blood pressure are reportedly the only effective treatment for intracranial hemorrhages (ICHs) (3).

In addition to the limited treatment strategies for hemorrhagic stroke, the administration of antiplatelet drugs and anticoagulants that is popular in clinical situations can increase the risk of hemorrhagic complications. For instance, it has been reported that one third of patients with ICH were administrated antiplatelet drugs (4).

The blood-brain barrier (BBB) is composed of a basement membrane, endothelial cells, pericytes, and tight junction proteins and plays an important role as a part of the neurovascular unit (5). Protection of the BBB is expected to be an important strategy for developing neuroprotective drugs (6). However, it remains unclear whether protecting the BBB can help reduce hemorrhagic stroke.

Before the CSPS 2 and meta-analysis were published, we focused mainly on examining the protective effects of cilostazol against brain ischemia; accordingly, we demonstrated the
protective effects of cilostazol against HT after transient cerebral ischemia (7). Based on previous studies, we hypothesized that cilostazol would be able to suppress hemorrhagic stroke by protecting the BBB, and we tested this hypothesis using various hemorrhagic stroke models. Here, we review what is known about the pleiotropic effects of cilostazol, report on the results we obtained using the various stroke models, and provide some suggestions for using cilostazol in the future (see Table 1).

2. Pleiotropic effects of cilostazol in basic research

2.1. Protection of endothelial cells

Many reports have demonstrated the protective effects of cilostazol on endothelial cells. For instance, cilostazol was shown to exert its protective effects on endothelial cells by increasing endothelial nitric oxide synthase activity (8). Moreover, cilostazol exerted protective effects against oxidative stress induced-endothelial senescence and dysfunction via the upregulation of Sirt1 (9). Oyama et al. reported that cilostazol reduced ischemic brain injury by protecting endothelial cells in spontaneously hypertensive rats (10). Collectively, these results indicate that cilostazol protects endothelial cells from damage in stroke.

2.2. Effects on vascular smooth muscle cells

Recently, it was reported that cilostazol prevents endothelin-induced smooth muscle cell constriction and proliferation (11). Except for its effect on vasoconstriction, cilostazol reportedly inhibits the abnormal proliferation of vascular smooth muscle cells (12). These results indicate that cilostazol also has a protective effect on vascular smooth muscle cells.

2.3. Protection of the BBB

Cilostazol also appears to have protective effects on the BBB. In vivo studies indicated that cilostazol exerts its neuroprotective effects in cerebral infarction by protecting the BBB (7,13). Omote et al. demonstrated that the administration of cilostazol reduced the spontaneous infarct volume and preserved motor and spatial cognitive functions in stroke-prone spontaneously hypertensive rats (14). These results suggest that cilostazol has the potential to protect the BBB and exert pleiotropic effects on stroke.

Based on many basic research studies, cilostazol is expected to protect endothelial cells, vascular smooth muscle cells, and the entire BBB from various types of damage. It is suggested that protection of the BBB could result in decreased hemorrhagic stroke, however, only a few studies on the protective effects of cilostazol against hemorrhagic stroke exist.

3. Recent clinical studies with cilostazol

As mentioned above, the CSPS 2 trial demonstrated that cilostazol is more effective than aspirin in the secondary prevention of all types of stroke, especially secondary hemorrhagic stroke (1). Cilostazol can also reduce stroke recurrence, poststroke ICH, and extracranial bleeding according to a meta-analysis (2). Additionally, a recent study showed that cilostazol exhibited beneficial effects on the outcome of patients with small vessel infarction (15).

A randomized clinical trial that compared the effects of cilostazol plus aspirin vs. aspirin alone on the progression of intracranial arterial stenosis (IAS) was published at 2015 (CATHARSIS). In that trial, no significant differences in IAS progression were observed between the two groups because the progression of IAS appears to be less frequent. However, the annual incidence rates of angio-
all vascular events, stroke, and ischemic stroke tended to be lower in the cilostazol plus aspirin group than in the group treated with aspirin alone. The results of the CATHARSIS trial suggest the potential utility of developing pharmacotherapies that combine cilostazol plus aspirin for managing symptomatic IAS (16).

Cilostazol has also attracted attention with regards to its potential to prevent restenosis in clinical situations. Cilostazol reduces angiographic restenosis after endovascular therapy for femoropopliteal lesions (17). In coronary disease, the addition of cilostazol to conventional dual antiplatelet therapy reportedly reduces the risk of cardiac events and restenosis after drug-eluting stent implantation (18).

In summary, cilostazol was better at reducing hemorrhagic stroke compared to aspirin. However, recent clinical reports focused on the effects of cilostazol on in-stent restenosis and found that the effects resulted from endothelial protection and the inhibition of vascular smooth muscle proliferation. Currently, no clinical trials have focused on the inhibitory effects of cilostazol on hemorrhagic stroke; however, this type of clinical trial is needed to confirm the protective effects of cilostazol against hemorrhagic stroke.

4. Protective effects of cilostazol against tPA-induced HT

Accumulating evidence suggests that thrombolysis is beneficial for patients with ischemic stroke if it is administered during the first 4.5 h after symptom onset; however, delayed thrombolysis was related to an increased risk of HT, which aggravated the clinical outcome in patients with acute ischemic brain attacks. Currently, there are no approved treatments for tPA- associated HT. Edaravone has been reported to have the potential to protect the BBB from ischemia-reperfusion injury and tPA toxicity (19), but has not been established as a treatment for hemorrhagic complications. Therefore, in a previous publication, we evaluated whether cilostazol would prevent tPA-associated HT (20).

In that study, mice subjected to 6-h middle cerebral artery occlusion (MCAO) were treated with delayed intravenous tPA alone, tPA plus cilostazol, or vehicle before reperfusion. We evaluated the histological changes in the hemorrhage, performed hemoglobin assays to measure the hemorrhage volume, and determined the level of matrix metalloproteinase-9 (MMP-9) activation.

The results showed that HT was significantly induced by tPA administration 6 h after the onset of ischemia. The combination of tPA plus cilostazol at 6 h significantly ameliorated the severity of HT (Fig. 1A, B). The MMP-9 activity was significantly decreased by tPA plus cilostazol at 6 h (Fig. 1C). These results indicated that cilostazol prevented the HT that was induced by focal cerebral ischemia in mice treated with tPA by protecting the endothelial cells and reducing MMP-9 (20).

Kasahara et al. also reported that treatment with cilostazol for 7 days before ischemia significantly suppressed the risk and severity of cerebral hemorrhaging after the injection of tPA and suppressed the microvasculature disruption in the ischemic area that is associated with reduced MMP-9 activity (21).

It is well known that MMP-9 activation causes the deterioration of tight junctions, which enhances the permeability and decreases the structural integrity of the BBB (22). It has also been suggested that MMP-9 activation in endothelial cells injured by ischemia-reperfusion is a mechanism underlying tPA-induced HT (23). In addition to these previous reports, the two results (20,21) mentioned above suggest that tPA itself is neurotoxic and results in impairment of the endothelial cells and tight junction proteins through MMP-9 activation. Such impairment of the endothelial cells and tight junction proteins causes BBB disruption; however, cilostazol has the potential to prevent this BBB disruption by suppressing MMP-9 activation in injured endothelial cells (Fig. 1D).

5. Protective effects of cilostazol against warfarin-induced HT

Warfarin is widely used to prevent cardio-embolic ischemic stroke, but it has the potential to exacerbate HT after cerebral ischemia. Previously, we investigated whether cilostazol is capable of suppressing warfarin-induced HT after cerebral ischemia in mice (24).

Mice were treated with oral warfarin before a 3-h MCAO, which was followed by a 21-h reperfusion to induce HT. The brain hemoglobin content was evaluated 24 h after MCAO. We also

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**Fig. 1.** The effect of cilostazol against tPA-induced HT. Modified from Ishiguro et al. (20) Histologic evaluation (A) and quantification of hemoglobin using hemoglobin assays at 24 h after MCAO (B). Data are expressed as the mean ± the standard deviation (SD). *p < 0.05 vs. vehicle, #p < 0.05 vs. tPA, Student's t-test, n = 7−10. (C) Result of the MMP-9 activity assay at 24 h after MCAO. Data are expressed as the mean ± SD. *p < 0.05 vs. vehicle, #p < 0.05 vs. tPA, Student's t-test, n = 7−10. (D) Proposed mechanism of cilostazol against tPA-induced HT.
evaluated the proteins involved in vascular endothelial integrity using western blotting. The results showed that the HT volume was exacerbated by warfarin treatment and that cilostazol suppressed this exacerbation (Fig. 2A). Furthermore, cilostazol upregulated the expression of tight junction proteins and vascular endothelial cadherin (VE-cadherin) (Fig. 2B). These findings indicated that cilostazol reduced the warfarin-related risk of HT after ischemia by protecting the VE-cadherin and tight junction proteins. In this study, MMP-9 was not increased by warfarin treatment, but cilostazol protected tight junction proteins and VE-cadherin (24).

Unlike tPA-induced HT, activation of MMP-9 was not detected in warfarin-induced HT. The deterioration of tight junction proteins and VE-cadherin was exacerbated by warfarin treatment, and was thus suggested as a mechanism of warfarin-induced HT. Cilostazol could prevent warfarin-induced hemorrhagic stroke by protecting tight junction and adherence junction proteins (Fig. 2C). These findings suggest that cilostazol reduces hemorrhage volume by exerting protective effects on vascular endothelial cells.

6. Cilostazol protects against collagenase-induced ICH by protecting the BBB

Based on the results of the two abovementioned examinations (20,24), we evaluated the effects of cilostazol pre-treatment on the collagenase-induced ICH volume and BBB permeability in mice in a previous publication (25). We also evaluated various vascular components in vitro, including endothelial cells, vascular smooth muscle cells, pericytes, and a co-culture model. Our results showed that pre-treatment with cilostazol reduced the ICH volume compared to vehicle (Fig. 3A). BBB permeability was increased by collagenase-induced ICH, and cilostazol attenuated the BBB leakage (Fig. 3B). Electron microscope images showed that the coverage rate of pericytes for endothelial cells was

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**Fig. 2.** The effect of cilostazol on warfarin-induced HT. Modified from Kitashoji et al. (24). (A) Effects of warfarin and cilostazol on cerebral hemorrhaging in mice at 21 h after reperfusion after MCAO. Brain histology using 40 μm-thick unstained cryosections (upper), and the effect of warfarin and cilostazol on hemorrhage volume (lower). Data are expressed as the mean ± SD. **p < 0.01 vs. vehicle, #p < 0.05 vs. warfarin, Tukey’s t-test; sham: n = 6, vehicle: n = 9, warfarin: n = 14, warfarin + cilostazol 1 mg/kg: n = 9, warfarin + cilostazol 3 mg/kg: n = 15. (B) Western blot analysis of claudin-5 and VE-cadherin at 6 h after reperfusion. Data are expressed as the mean ± the standard error of the mean (SEM) *p < 0.05, **p < 0.01 vs. sham; #p < 0.05, ##p < 0.01 vs. vehicle; $$p < 0.01 vs. warfarin, Student’s t-test; sham: n = 9, vehicle: n = 9; warfarin: n = 9, warfarin + cilostazol 3 mg/kg: n = 8. WR, warfarin; CSZ, cilostazol. (C) Suggested mechanism of cilostazol against warfarin-induced HT.
improved by the cilostazol administration (Fig. 3C). In vitro experiments showed that cilostazol prevented both endothelial cells and pericytes from collagenase-induced cell death. Cilostazol also protected tight junction proteins, adherence junction proteins, and the basement membrane from collagenase-induced injury according to western blot analyses (25). Therefore, cilostazol likely reduces the collagenase-induced ICH volume by protecting the entire BBB (Fig. 3D).

Fig. 3. The effect of cilostazol on collagenase-induced ICH. Modified from Takagi et al. (25). (A) A representative hemorrhage from each group is shown in the upper panel. The bottom graph shows the hematoma volume 24 h after hemorrhage induction as measured by hemoglobin quantification. *p < 0.05 vs. vehicle, #p < 0.05 vs. aspirin, Student’s t-test, n = 18–19. (B) Permeability of fluorescein isothiocyanate (FITC)-dextran 3 h after ICH induction. The images in the upper panel show typical FITC leakage, with the green color indicating FITC-dextran. The graph on the right shows the results of the statistical analysis of the FITC leakage. *p < 0.05, **p < 0.01 vs. control, Dunnet’s comparison test; #p < 0.01 vs. vehicle, Student’s t-test, n = 4 for each group. (C) Typical electron microscope image obtained after collagenase-induced ICH showing the pericyte coverage to endothelial cell ratio. Statistical analysis of pericyte coverage was on the right. The black arrow indicates a pericyte. Scale bar = 500 nm **p < 0.01 vs. control, ##p < 0.01 vs. vehicle, Student’s t-test, n = 3–4. (D) Proposed mechanism of cilostazol against collagenase-induced ICH.
Collagenase impairs endothelial cells, pericytes, and other BBB components like the basement membrane and tight junction proteins. The ability of cilostazol to decrease hematoma size is likely a result of its protective effects on both endothelial cells and pericytes. Cilostazol is thought to activate cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) phosphorylation through cAMP/protein kinase A signaling and protects both endothelial cells and pericytes from collagenase-induced cell death, which may result in increased extracellular matrix.

In addition to our report, Torii et al. indicated that cilostazol prevented an increase in the endothelial permeability by preserving the actin cytoskeleton and the redistribution of junctional proteins in hypoxia/reoxygenation (26). Collectively, previous reports on the protective effects of cilostazol on the BBB support that cilostazol can protect all components of the BBB, thus reducing the ICH volume. Such findings suggest that cilostazol has the potential to reduce hemorrhagic stroke, as predicted in clinical trials.

7. Future perspectives of cilostazol

7.1. Effects of cilostazol on vascular dementia

Cilostazol reportedly has the potential to suppress cognitive decline in patients with mild dementia who are receiving donepezil (27). After the publication of that report, other studies began examining the effects of cilostazol on vascular dementia. A study by Kumar et al. investigated the potential applications of cilostazol for diabetes-induced vascular dementia by using streptozotocin to induce diabetes and subsequent vascular dementia in Wistar rats. They found that treatment with cilostazol significantly attenuated the diabetes-induced learning and memory impairments and endothelial dysfunction (28).

Moreover, a study by Kwon et al. tested whether cognitive impairment could be exacerbated in a combined injury by using a model of chronic cerebral hypoperfusion with diabetes. They also investigated the protective effects of cilostazol against cognitive impairment. The authors demonstrated that there are deleterious interactions between chronic cerebral hypoperfusion and type II diabetes mellitus and found that cilostazol ameliorated the cognitive impairments in rats with diabetes mellitus and hypoperfusion-induced dementia (29).

These results indicated that cilostazol could improve the memory impairments directly through inhibition of neuronal cell death via the upregulation of phospho-CREB and brain-derived neurotrophic factor; moreover, cilostazol could indirectly attenuate the learning and memory impairments by improving endothelial dysfunction.

7.2. Effects of cilostazol on cerebral beta amyloid drainage

Maki et al. reported that cilostazol rescued the cognitive deficits in a transgenic mouse (Tg-SwDI mice) model of cerebrovascular beta-amyloidosis. Additionally, they demonstrated that cilostazol treatment maintained cerebral hyperemic and vasodilative responses, suppressed the degeneration of pericytes and vascular smooth muscle cells, and promoted the perivascular drainage of soluble Aβ1–40 (30). These results suggest that cilostazol might be a candidate for suppressing cognitive impairment in Alzheimer’s disease or vascular dementia and imply that these neuroprotective effects were exerted through protection of the BBB.

Cilostazol can protect endothelial cells, vascular smooth muscle cells, pericytes, the basement membrane, tight junction proteins, and adherence junction proteins from various types of damage. As a result of its multiple effects, cilostazol can suppress hemorrhagic stroke by protecting all of the components of the BBB. A clinical trial that focuses on the ability of cilostazol to suppress bleeding is needed to apply the results of basic research studies to clinical practice. The effects of cilostazol on stroke may also be extended to cognitive impairments in the future.

Conflicts of interest

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References


