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Current perspective

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

Toshinori Takagi [a, b], Hideaki Hara [a, *]

[a] Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu, Japan
[b] Department of Neurosurgery, Gifu Prefectural Gero Hot Spring Hospital, 2211 Mori, Gero, Japan

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ABSTRACT

Cilostazol is a phosphodiesterase-3 inhibitor and is known to have pleiotropic effects including antiplatelet 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.

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 study and meta-analysis were published, we focused mainly on examining the protective effects of cilostazol against brain ischemia; accordingly, we demonstrated the

* Corresponding author.
E-mail address: hidehara@gifu-pu.ac.jp (H. Hara).
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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 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

Table 1
Pleiotropic effects of cilostazol.

Target	Effect	Animal/Cell	Model	Mechanism	Reference
Endothelial cell	Endothelial cell protection	Human endothelial cells	High glucose	NO production	8
		Human endothelial cells	Hydrogen peroxide	Upregulation of Sirt1	9
		Spontaneously hypertensive rats	Spontaneous hypertension	Increase of phospho-eNOS	10
Vascular smooth muscle cell	Protection of vascular smooth muscle	VSMC, Basilar artery (ex vivo)	Endothelin-induced vascular constriction and vascular proliferation	Blocking extracellular calcium influx	11
		VSMC	Serum-induced mitogenesis	Reduction of phosphorylated ERK1/2	12
Blood-brain barrier	Reduction of cerebral infarction	Rat	Transient MCAO	Inhibition of the apoptotic pathway and oxidative stress	13
	Reduction of cerebral infarction	Stroke-prone spontaneously hypertensive rats	Spontaneous stroke	Reduction of oxidative stress	14
	Reduction of tPA-induced HT	Mouse	Transient MCAO + tPA	Reduction of MMP-9	20,21
	Reduction of warfarin-induced HT	Mouse	Warfarin + transient MCAO	Protection of TJP and VE-cadherin	24
	Reduction of ICH volume	Mouse	Collagenase-induced ICH	Increase of phospho-CREB	26
	Improvement of learning and memory impairment in vascular dementia	Rat	Streptozotocin induced diabetes and vascular dementia	Protection of endothelial dysfunction	28
	Improvement of learning and memory impairment in vascular dementia	Rat	Cerebral hypoperfusion and type II DM		29
	Auppression of cognitive impairment in Alzheimer's disease	Tg-SwDI mice	Cerebrovascular beta-amyloidosis	Promotion of the perivascular drainage of soluble Abeta1-40	30

CREB: cAMP response element binding protein, DM: diabetes mellitus, ERK: extracellular signal-regulated kinase, HT: hemorrhagic transformation, ICH: intracranial hemorrhage, MCAO: middle cerebral artery occlusion, MMP-9: matrix metalloproteinase-9, NO: nitric oxide, eNOS: endothelial nitric oxide synthase, TJP: tight junction protein, VE-: vascular endothelial, VSMC: vascular smooth muscle.