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ORIGINAL ARTICLE

Dose-Dependent Inhibitory Effects of Cilostazol on Delayed Cerebral Infarction After Aneurysmal Subarachnoid Hemorrhage

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Abstract

Cilostazol is a selective inhibitor of phosphodiesterase type III that downregulates tenascin-C (TNC), a matricellular protein, which may cause delayed cerebral infarction after aneurysmal subarachnoid hemorrhage (SAH). The authors increased the dosage and evaluated the dose-dependent effects of cilostazol on delayed cerebral infarction and outcomes in SAH patients. This was a retrospective cohort study in a single center. One hundred fifty-six consecutive SAH patients including 67 patients of admission World Federation of Neurological Surgeons grades IV–V who underwent aneurysmal obliteration within 48 h post-SAH from 2007 to 2017 were analyzed. Cilostazol (0 to 300 mg/day) was administered from 1-day post-clipping or post-coiling to day 14 or later. Cilostazol treatment dose-dependently decreased delayed cerebral infarction and tended to improve outcomes, although cilostazol did not affect other outcome measures including angiographic vasospasm. On multivariate analyses, 300 mg/day (100 mg three times) cilostazol independently decreased delayed cerebral infarction and improved 3-month outcomes, but other regimens including 200 mg/day (100 mg twice) cilostazol were not independent prognostic factors. Propensity score-matched analyses showed that the 300 mg/day cilostazol cohort had lower plasma TNC levels and a lower incidence of delayed cerebral infarction associated with better outcomes compared with the non-cilostazol cohort. The 300 mg/day cilostazol may improve post-SAH outcomes by reducing plasma TNC levels and delayed cerebral infarction, but not vasospasm. Further studies are warranted to investigate if 300 mg/day cilostazol is more beneficial to post-SAH outcomes than a usual dose of 200 mg/day cilostazol that was demonstrated to be effective in randomized controlled trials.

Keywords Cerebral infarction · Cerebral vasospasm · Cilostazol · Delayed cerebral ischemia · Subarachnoid hemorrhage · Tenascin-C

Introduction

Aneurysmal subarachnoid hemorrhage (SAH) remains a devastating cerebrovascular disease [1]. Patients who survive early brain injury (EBI), deleterious effects of acute SAH on brain [2], and have their aneurysms secured by clipping or coiling are still at risk for delayed cerebral infarction [3]. Non-iatrogenic cerebral infarction on neuroimaging may be a better outcome measure than clinical deterioration caused by delayed cerebral ischemia (DCI) [3]. However, SAH-induced cerebral infarction is a challenging problem involving different mechanisms such as EBI and cerebral vasospasm [1–4].

Tenascin-C (TNC), a matricellular protein, may cause EBI, cerebral vasospasm, and DCI after SAH [5–10]. Plasma levels of large-splice variants of TNC containing C domain in the fibronectin type III (FNIII) repeats transiently increased a few days before the onset of DCI after SAH [11]. Cilostazol, a selective inhibitor of phosphodiesterase type III, is a clinically available anti-platelet with pleiotropic actions and can inhibit TNC induction at the transcriptional level [12]. Cilostazol prevented post-SAH angiographic vasospasm (aVSP) in rats [13, 14], and a meta-analysis of recent randomized controlled trials (RCTs) reported that cilostazol was the only pharmacological intervention

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to improve outcomes after SAH [15]. According to previous RCTs, however, a daily usual dose of cilostazol (200 mg/day, 100 mg twice a day) significantly decreased some of aVSP, DCI, cerebral infarction, and poor outcome [16–18], but the effects were limited especially in poor-grade SAH patients [16]. Moreover, the studies did not include the worst clinical grade V patients [16–18], although delayed cerebral infarction reportedly occurred in about 65% of the grade V patients with aneurysmal obliteration even in the current era [19]. Because cilostazol's effects are dose-dependent with the time to peak blood concentration of 3 to 4 h and half-lives of about 3 h [20, 21], the authors hypothesized that higher dosages of cilostazol should more effectively prevent post-SAH delayed cerebral infarction and have gradually increased the daily dosages from 0 to 300 mg (100 mg three times, every 8 h) for fear of the hemorrhagic complications. The aims of this retrospective study were thus to evaluate dose-dependent effects of cilostazol on delayed cerebral infarction and outcomes in an aneurysmal SAH cohort including many poor-grade patients and, especially, to assess if 300 mg/day (100 mg three times) cilostazol had the additional beneficial effects compared with a usual dose of 200 mg/day (100 mg twice) cilostazol. We also measured plasma TNC levels as a biomarker for delayed cerebral infarction and eventual poor outcomes and compared the values between non-cilostazol and 300 mg/day cilostazol treatment groups.

Materials and Methods

Patient Population

A prospectively maintained SAH database at our institution was searched for patients who underwent clipping or coiling for ruptured cerebral aneurysms within 48 h after onset between January 2007 and August 2017. Medical records of 156 consecutive patients (40 males and 116 females), 25 to 90 years of age (mean 63.6 years), were retrieved and retrospectively reviewed. The ethical committee of our institute approved this study and waived the need for informed consent. The inclusion criteria were ≥ 20 years of age at onset, SAH on computed tomography (CT) scans, saccular aneurysm as the cause of SAH confirmed on digital subtraction angiography (DSA), and aneurysmal obliteration within 48 h of onset. Excluded from the study were patients with dissecting, traumatic, mycotic, and arteriovenous malformation-related aneurysms or SAH of unknown etiology. The World Federation of Neurological Surgeons (WFNS) grade at admission included 89 patients of grades I–III, and 67 patients (42.9%) of grades IV and V [22]. The ruptured aneurysm location was the anterior communicating artery in 39 patients, internal carotid artery in 51, middle cerebral artery in 29, vertebral or basilar arteries in 23, and other arteries in 14.

Aneurysmal clipping (48 patients) or coiling (108 patients) was performed as judged by the attending neurosurgeons to be appropriate for the individual patient. A ventricular catheter was placed in all patients with ventriculomegaly and a decreased level of consciousness that could not be attributed to causes other than acute hydrocephalus at admission. Cisternal drainage was placed in the basal cistern after clipping, and lumbar spinal drainage was placed after coiling to promote SAH clearance according to the preference of attending neurosurgeons. If progressive ventriculomegaly was observed post-operatively and within 14 days of onset, lumbar spinal drainage was placed irrespective of clipping or coiling. All patients without renal dysfunction received intravenous fasudil hydrochloride (Asahi Kasei Pharma Co., Tokyo, Japan) from 1-day post-clipping or post-coiling to day 14 post-hemorrhage. Additional treatments were included to administer sufficient intravenous fluid volume and enough nutrition; to prevent meningitis, pneumonia, and hypoxia; and to correct anemia and hypoproteinemia.

Cilostazol Treatment

Oral or enteral cilostazol (Otsuka Pharmaceutical Co., Tokyo, Japan) was administered from 1-day post-clipping or post-coiling to day 14 or later. A daily dosage was 100 mg (50 mg twice), 150 mg (50 mg three times), or 200 mg (100 mg twice) between January 2007 and September 2013 according to the preference of attending neurosurgeons. From October 2013, the 300 mg/day cilostazol protocol, which was approved by the ethical committee of our institute, was employed: 100 mg/time cilostazol was administered twice a day (every 12 h) from 1-day post-clipping or post-coiling to day 3, increased to three times a day (every 8 h) from days 4 to 10, and returned to twice a day from days 11 to 14 or later.

Outcome Measures

Primary outcome measures were incidences of delayed cerebral infarction and functional outcomes. Delayed cerebral infarction was defined as a new infarct on CT scans that was not visible on the day following operation or intervention. All patients underwent CT scans at admission, on the day post-operation or post-intervention, every 4–5 days until day 14, and then once a week until discharge. The outcome was evaluated using the modified Rankin Scale (mRS) at discharge and 3 months post-SAH.

Secondary outcome measures included incidences of DCI, aVSP, chemical angioplasty (intra-arterial fasudil hydrochloride infusions), percutaneous transluminal angioplasty (PTA), shunt-dependent hydrocephalus, and seizure. DCI was defined as any neurological deterioration presumed related to ischemia that persisted for ≥ 1 h independent of imaging

findings, after rigorous exclusion of other potential causes of clinical deterioration [3]. DCI was treated with hypertensive hypervolemic therapy with or without chemical angioplasty and/or PTA. aVSP was defined as ≥ 50% reduction in the baseline vessel diameter of the internal carotid artery, A1-2, M1-2, P1-2 segments (main trunk of anterior, middle or posterior cerebral arteries), or vertebrobasilar artery demonstrated by DSA, three-dimensional CT angiography, or magnetic resonance angiography, which were performed at least at days 6–8, days 15–21, and at the onset of clinical symptoms. Chronic hydrocephalus was diagnosed when a clinical deterioration with no detectable causes other than hydrocephalus occurred after day 14, and the ventricular size progressively increased (Evans index ≥ 0.30) [23]. Chronic hydrocephalus was treated with ventriculoperitoneal shunting (shunt-dependent hydrocephalus).

Measurement of TNC

We previously measured plasma TNC levels in SAH patients with no cilostazol treatment [11]. Plasma TNC levels were also measured in SAH patients treated with the 300 mg/day cilostazol protocol this time. Given that patients were not randomized to receive 300 mg/day cilostazol, propensity score matching was performed to balance selected pretreatment variables between the two treatment arms after excluding patients with diseases that could affect TNC metabolism [11]. The institutional ethics committee approved the study, and written informed consent was obtained from their relatives.

After CT confirmation of no iatrogenic complications on the day post-operation or post-intervention, blood samples were collected with minimal stasis from a vein on days 1–3, 4–6, 7–,9 and 10–12 post-SAH. Control samples were obtained from seven patients with unruptured cerebral aneurysms. All samples were centrifuged for 5 min at 3000×*g*, and supernatants were stored at − 80 °C until assayed. TNC concentrations were determined using ELISA kits for human large-splice variants of TNC containing FNIII C domain (IBL, Takasaki, Japan).

Statistical Analysis

Categorical variables were reported as a percentile and analyzed using chi-square or Fisher's exact test, as appropriate. For multiple comparisons of proportions, adjusted *P* values were calculated based on the method by Benjamini and Hochberg [24] using R (version 3.5.0) statistical software. For other statistical analyses, commercially available software (SPSS version 24, IBM Corp.) was used. Continuous variable (age) was expressed as means ± standard deviation and/or medians (interquartile ranges). After confirming that each population being compared followed a normal distribution using Shapiro-Wilk *W* tests, statistical differences were analyzed using unpaired *t* tests or one-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparison procedure. Multivariate unconditional logistic regression models were used to find independent association with delayed cerebral infarction or good outcomes (3-month mRS 0–2) after controlling for all variables regardless of the significance on univariate analyses. Adjusted odds ratios with 95% confidence intervals were calculated, and independence of variables was tested using the likelihood ratio test on reduced models. A P value < 0.05 was considered significant.

Propensity score matching was performed using a multivariable logistic regression model, with exposure to 300 mg/day cilostazol treatment as the dichotomous treatment variable, and with respect to age, sex, admission WFNS grade, Fisher's CT group, acute hydrocephalus, aneurysm location, aneurysm obliteration day, treatment modality (clipping or coiling), and cerebrospinal fluid (CSF) drainage. After propensity score generation, patients underwent 1:1 nearest-neighbor matching of the logit of the propensity score with a caliper width of 0.2 of the standard deviation of the score [25]. Matching was performed without replacement, with unpaired-treated and control patients not meeting matching criteria being excluded. Imbalance of the covariate was defined as the absolute standardized difference value ≥ $1.96 \times \sqrt{2}/n$ [26]. The primary and secondary outcome measures as well as TNC values after propensity score matching were compared between the two groups.

Results

Baseline Characteristics

Forty-nine patients were treated with no cilostazol, while 12, 6, 38, and 51 patients were treated with 100 mg/day, 150 mg/day, 200 mg/day, and 300 mg/day cilostazol, respectively. The 300 mg/day cilostazol group had more severe SAH (higher incidences of Fisher's CT group 3, and lower incidences of modified Fisher's CT grade 1) [27] even compared with the 200 mg/day cilostazol group, but baseline treatment characteristics were not different among the groups (Online Resource Tables S1, S2). Patients treated with 150 mg/day cilostazol more likely had ruptured middle cerebral artery aneurysms.

Cilostazol Treatment and Outcomes

Cilostazol dose-dependently decreased the incidence of delayed cerebral infarction: the differences between 0 or 100 mg/day and 300 mg/day cilostazol reached statistical significance ($P < 0.005$ and $P < 0.05$, respectively, adjusted *P* value based on Benjamini and Hochberg; Fig. 1). The incidence of delayed cerebral infarction in the 300 mg/day

cilostazol group was also significantly lower compared with the 200 mg/day cilostazol group on Fisher's exact tests ($P < 0.05$), but this significance was lost after correction for multiple comparisons (Fig. 1). The 300 mg/day cilostazol also tended to increase 3-month good outcomes (mRS 0–2; Table 1), but failed to decrease other secondary outcome measures including the overall incidence of aVSP even compared with no cilostazol (Table 2).

Multivariate analyses revealed that both 200 mg/day and 300 mg/day cilostazol treatments independently suppressed delayed cerebral infarction, even though either all baseline characteristics or all variables except for primary outcome measures were used as variables (Online Resource Tables S3, S4). However, 3-month good outcomes were independently increased only by 300 mg/day cilostazol, but not by other cilostazol regimens including 200 mg/day cilostazol (Table 3).

Plasma TNC Levels Following Propensity Score Adjustment

Equal numbers of non-cilostazol and 300 mg/day-cilostazol patients ($n = 24$ per group) were matched, and all covariates were statistically indistinguishable between the cohorts (Online Resource Table S5): the number of excluded patients was 7 and 27, respectively. Matched cohorts exhibited significantly lower incidences of delayed cerebral infarction and higher incidences of 3-month good outcomes when patients were treated with 300 mg/day cilostazol compared with those without (Online Resource Table S6). However, there were no significant differences between the cohorts with respect to secondary outcomes.

Plasma TNC levels at days 1–3 post-SAH in the non-cilostazol cohort significantly increased compared with patients with unruptured cerebral aneurysms (140.3 ± 84.9 versus 39.2 ± 3.5 ng/mL; $P < 0.0001$, ANOVA), but the values in the 300 mg/day cilostazol cohort (41.2 ± 21.1 ng/mL) were similar to the control values. Plasma TNC levels were significantly lower in the 300 mg/day cilostazol cohort compared with non-cilostazol cohort during the observation period (Fig. 2a). Plasma TNC levels in patients with DCI, an independent determinant for both delayed cerebral infarction (Online Resource Table S4) and poor outcomes (Table 3), were significantly higher than those without DCI at days 4–6 post-SAH (Fig. 2b).

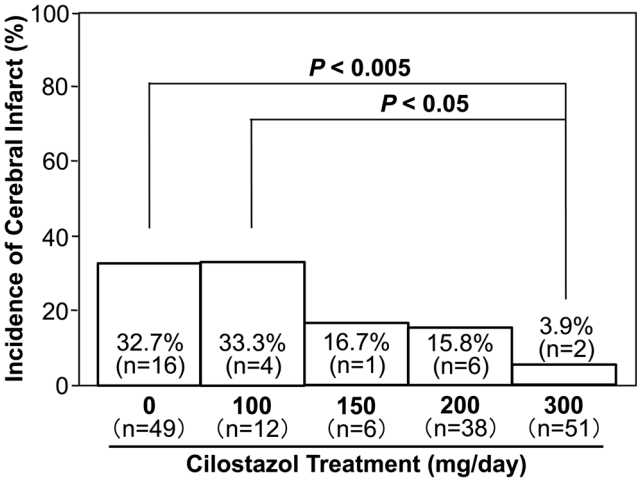

Fig. 1 The incidence of delayed cerebral infarction after subarachnoid hemorrhage in 0 to 300 mg/day cilostazol treatments. *P* values, adjusted *P* value based on Benjamini and Hochberg

Adverse Effects of Cilostazol

As to tolerability, 150 mg/day cilostazol was discontinued due to headache in one patient (16.7%); 200 mg/day cilostazol caused discontinuation due to sinus tachycardia in one patient (2.6%) and dose-down due to pleural effusion in one patient (2.6%); and 300 mg/day cilostazol caused transient discontinuation due to heart failure and pleural effusion in one patient (2.0%), headache and sinus tachycardia in one patient (2.0%), and discontinuation due to sinus tachycardia in one patient (2.0%). All these complications improved with symptomatic treatment. Neither hemorrhagic complications needing treatment nor intracranial bleeding detectable on CT scans occurred. Headache, which might be aggravated by cilostazol, was well-controlled with analgesics in all patients except for the above two patients.

Discussion This study first demonstrated that 1-week cilostazol dose-up (300 mg/day) treatment was safe, and independently decreased aVSP-unrelated delayed cerebral infarction and improved 3-month outcomes associated with suppression of plasma TNC levels. More importantly, this study suggested that 300 mg/day cilostazol may be more effective to improve post-SAH outcomes than a usual dose of 200 mg/day cilostazol, of which the efficacy was demonstrated in recent RCTs.

Cilostazol is an anti-platelet drug and a peripheral artery vasodilator with pleiotropic actions that has been clinically used for the treatment of ischemic symptoms in chronic peripheral arterial obstruction and for the prevention of recurrent cerebral infarction in Japan [13]. Experimental studies demonstrated that cilostazol prevented post-SAH aVSP by inhibiting lipid peroxidation [14], inflammatory reactions [13], and endothelial damage and phenotypic changes of cerebral arteries [28]. An in vitro study also reported that cilostazol inhibited the induction of TNC [12], which has been demonstrated to cause post-SAH EBI, aVSP, and DCI using TNC knockout mice and recombinant TNC [5–10]. However, two small-scale RCTs, which were conducted for aneurysmal SAH patients treated with clipping, reported conflicting results: in one study, 200 mg/day cilostazol did not decrease DCI nor delayed cerebral infarction, but improved outcomes [18], while another study reported that 200 mg/day cilostazol

Table 1 Modified Rankin scale after different dosages of cilostazol treatment in aneurysmal subarachnoid hemorrhage

Modified Rankin scale	Cilostazol treatment (mg/day)				
	0 ($n = 49$)	100 ($n = 12$)	150 ($n = 6$)	200 ($n = 38$)	300 ($n = 51$)
At discharge					
0	11 (22.4)	4 (33.3)	2 (33.3)	11 (28.9)	9 (17.6)
1	11 (22.4)	0	1 (16.7)	6 (15.8)	7 (13.7)
2	2 (4.1)	1 (8.3)	1 (16.7)	5 (13.2)	9 (17.6)
3	3 (6.1)	1 (8.3)	0	5 (13.2)	9 (17.6)
4	7 (14.3)	0	0	3 (7.7)	10 (19.6)
5	15 (30.6)	4 (33.3)	2 (33.3)	8 (21.1)	7 (13.7)
6	0	2 (16.7)	0	0	0
0–2	24 (49.0)	5 (41.7)	4 (66.7)	22 (57.9)	25 (49.0)
3 months after onset					
0	15 (30.6)	4 (33.3)	3 (50.0)	14 (36.8)	17 (33.3)
1	8 (16.3)	1 (8.3)	0	7 (18.4)	9 (17.6)
2	3 (6.1)	1 (8.3)	1 (16.7)	5 (13.2)	11 (21.6)
3	5 (10.2)	1 (8.3)	0	2 (5.3)	2 (3.9)
4	3 (6.1)	0	0	2 (5.3)	7 (13.7)
5	15 (30.6)	3 (25.0)	2 (33.3)	8 (21.1)	5 (9.8)
6	0	2 (16.7)	0	0	0
0–2	26 (53.1)	6 (50.0)	4 (66.7)	26 (68.4)	37 (72.5)

Data indicate number of case (% of total case per group). Adjusted *P* value based on Benjamini and Hochberg: no significant differences among groups

reduced aVSP, DCI, and delayed cerebral infarction, but did not improve outcomes [16]. In the third RCT, in which aneurysmal SAH patients were treated with either clipping or coiling, 200 mg/day cilostazol did not reduce aVSP nor delay cerebral infarction, but decreased DCI and poor outcomes [17]. Taken together, effects of a usual dose of cilostazol (200 mg/day) were not enough in a clinical setting, especially in poor-grade SAH patients [16]. In addition, the above three RCTs did not include the worst clinical grade patients. This study included 42.9% of the WFNS grades IV–V patients and showed that 300 mg/day cilostazol treatment was an independent suppressor of delayed cerebral infarction and poor outcomes, while 200 mg/day cilostazol did not improve outcomes independently.

Effects of cilostazol are reported to be dose-dependent [20]. An increase in cilostazol concentrations from 100 to 200 μM

Table 2 Secondary outcome measures after different dosages of cilostazol treatment in aneurysmal subarachnoid hemorrhage

Secondary outcome measures	Cilostazol treatment (mg/day)				
	0 ($n = 49$)	100 ($n = 12$)	150 ($n = 6$)	200 ($n = 38$)	300 ($n = 51$)
Delayed cerebral ischemia	6 (12.2)	2 (16.7)	0	4 (10.5)	9 (17.6)
Angiographic vasospasm[a]	15 (34.9)	2 (18.2)	2 (33.3)	7 (18.9)	13 (25.5)
On DSA[a]	10 (23.2)	1 (9.1)	1 (16.7)	5 (13.5)	4 (7.8)
On MRA[a]	5 (11.6)	1 (9.1)	1 (16.7)	2 (5.4)	9 (17.6)
Unknown[b]	6 (12.2)	1 (8.3)	0	1 (2.6)	0
Chemical angioplasty[c]	2 (4.1)	0	0	3 (7.9)	3 (5.9)
PTA	0	0	0	2 (5.3)	0
Shunt-dependent hydrocephalus	13 (26.5)	2 (16.7)	0	7 (18.4)	13 (25.5)
Seizure	3 (6.1)	1 (8.3)	0	0	1 (2.0)

Data indicate number of case (% of total case or [a] % of cases except for unexamined cases per group). [b] Cases that underwent neither digital subtraction angiography (DSA) nor magnetic resonance angiography (MRA) due to poor neurological or systemic conditions. [c] Intra-arterial injection of fasudil hydrochloride. *PTA*, percutaneous transluminal angioplasty. Adjusted *P* value based on Benjamini and Hochberg: no significant differences among groups

Table 3 Significant variables on multivariate logistic regression with 3-month good outcome (modified Rankin scale 0–2) as a binary end point

Variables	Odds ratio	95% confidence interval	*P* value
Age	0.796	0.714–0.886	< 0.001
300 mg/day cilostazol treatment	37.963	2.172–663.655	0.013
Admission WFNS grade II	37.044	2.041–672.458	0.015
Delayed cerebral ischemia	0.020	0.001–0.508	0.018
Shunt-dependent hydrocephalus	0.011	0.000–0.377	0.012

Age, sex, admission World Federation of Neurological Surgeons (WFNS) grade, Fisher's computed tomography group, acute hydrocephalus, aneurysm location, aneurysm obliteration day, treatment modality (clipping or coiling), cilostazol treatment, cerebrospinal fluid drainage, delayed cerebral ischemia, angiographic vasospasm, chemical angioplasty, percutaneous transluminal angioplasty, delayed cerebral infarction, shunt-dependent hydrocephalus, and seizure are used in multivariate logistic regression. Age is used as a continuous variable. References are as follows: no cilostazol treatment for cilostazol treatment and admission WFNS grade I for admission WFNS grade

inhibited the proliferation of cultured vascular smooth muscle cells to one tenth in a concentration-dependent manner [20]. A phase I study showed that a single administration of 25 to 300 mg cilostazol increased the blood concentration dose-dependently, which peaked after 3 to 4 h and declined with half-lives of about 3 h [21]. In another clinical study, 300 mg/day (100 mg three times) cilostazol was used for chronic arterial occlusive diseases of the extremities for 6 weeks, and the only side effect was well-controlled headache [29]. Our pilot study reported that 300 mg/day (100 mg three times) cilostazol was safe and feasible in post-operative SAH patients and, more effectively, suppressed both the plasma levels of TNC variants containing FNIII B domain and the development of chronic hydrocephalus compared with 100–200 mg/day cilostazol [30]. The previous studies support the present results that 300 mg/day (100 mg every 8 h) cilostazol was the best to improve outcomes among tested dosages of 0 to 300 mg/day cilostazol.

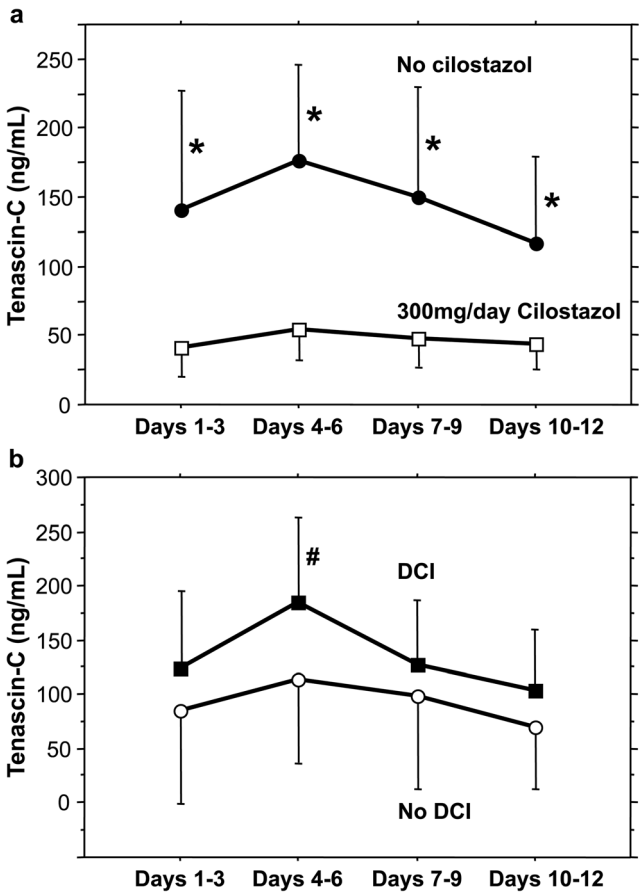

Fig. 2 Plasma tenascin-C concentrations at days 1 to 12 after subarachnoid hemorrhage. Comparison between patients treated with no cilostazol and 300 mg/day cilostazol (**a**) and between patients with and without delayed cerebral ischemia (DCI; **b**). Unpaired *t* tests: $^{*}P < 0.0001$ versus patients treated with 300 mg/day cilostazol; $^{\#}P = 0.0130$ versus patients with no DCI

EBI, acute pathophysiological events that occur in the brain before onset of cerebral vasospasm within the first 72 h of aneurysmal SAH [2], is clinically characterized by global cerebral edema and poor clinical grade [31]. EBI is now believed to be more important than cerebral vasospasm, a classically important determinant of poor outcome, in post-SAH outcomes [32]. EBI may also contribute to the development of delayed cerebral infarction, another important determinant of poor outcome [2, 3]. In this study, 300 mg/day cilostazol decreased delayed cerebral infarction and poor outcomes in the SAH cohort including 42.9% of the WFNS grades IV–V patients, but other outcome measures including aVSP and DCI were not improved. However, this study confirmed our previous study [11]: patients with DCI had higher plasma levels of TNC variants containing C domain (not B domain) at days 4–6 post-SAH compared with those without DCI. In this study, 300 mg/day cilostazol almost completely suppressed plasma TNC levels, but the inhibitory effects on CSF TNC levels were not measured. TNC variants containing C domain was also increased markedly in the CSF after SAH [33]. As the dosage of cilostazol to prevent aVSP was much higher (about 15 times) in a rat SAH model compared with clinical settings [14], further higher dosages of cilostazol might have needed to suppress CSF TNC and prevent aVSP. That is, 300 mg/day cilostazol might prevent cerebral infarction secondary to EBI, but not

vasospasm, by suppressing only plasma TNC induction. Thus, the optimal protocol including a dosage of cilostazol, the timing, and period of administration should be determined.

An ideal biomarker may be a molecule that is implicated in the pathogenesis of EBI and subsequent DCI, being a therapeutic target, and can be measured easily in the peripheral blood in an acute stage of SAH. A good candidate of such a biomarker is TNC [34]. Neuroinflammation is also a major aspect of EBI [35], but the evidence for cytokines or other inflammatory mediators as biomarkers is conflicting and inconsistent, possibly because various pathophysiological reactions other than EBI influence inflammatory reactions, and therefore, the specificity of inflammatory reactions for EBI may become low [36]. As well, many molecules such as neuron-specific enolase are released from neurons and/or glia into the peripheral blood after SAH and can be used as biomarkers reflecting the extent of EBI [37], but not be a therapeutic target. Plasma TNC levels peaked at days 4–6, and the peak occurred a few days before the occurrence of aVSP and DCI [11], while CSF TNC levels peaked immediately after SAH, and higher CSF TNC levels were associated with subsequent aVSP, DCI, and poor outcome [5, 33]. Although the differences in the time course of TNC levels between plasma and CSF may reflect the time lag that TNC is released from CSF into plasma due to its huge molecular weight, no studies have investigated whether peripherally detected TNC levels accurately reflect the levels in CSF [34]. As the need to collect CSF is associated with some invasiveness, however, plasma TNC may be a better biomarker to timely diagnose the development of aVSP and DCI at this time. However, it is also unknown if plasma TNC can detect the differences in therapeutic effects between 200 and 300 mg/day cilostazol.

There are some limitations in this study. First, although we administered fasudil hydrochloride, a Rho-kinase inhibitor, for the prevention of cerebral vasospasm [38], the Rho-kinase inhibitor also can suppress TNC expression [39]. However, it remains undetermined if the combination effects of fasudil and cilostazol on TNC expression and post-SAH pathophysiologies are additive, synergistic, or interactive, needing further studies. Second, a higher ratio of coiling in this study compared with previous clinical studies using cilostazol might influence outcomes. Lastly, this is a small-scale retrospective study with potential selection bias. To confirm the efficacy, therefore, large-scale RCTs are needed, especially to investigate if 300 mg/day cilostazol is more beneficial to post-SAH outcomes compared with 200 mg/day cilostazol.

Conclusions

This study showed that 300 mg/day cilostazol is safe and promising to reduce delayed cerebral infarction and to improve outcomes after aneurysmal SAH and is worthy of further studies especially to compare the therapeutic effects of 300 mg/day cilostazol with those of 200 mg/day cilostazol, a regimen that was demonstrated to be effective in RCTs.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study as to TNC measurements. For other retrospective analyses, formal consent is not required.

References

1. Macdonald RL, Schweizer TA. Spontaneous subarachnoid haemorrhage. Lancet. 2017;389:655–66.
2. Suzuki H. What is early brain injury? Transl Stroke Res. 2015;6:1–3.
3. Vergouwen MDI, Vermeulen M, van Gijn J, Rinkel GJE, Wijdicks EF, Muizelaar JP, et al. Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. Stroke. 2010;41:2391–5.
4. Suzuki H, Shiba M, Nakatsuka Y, Nakano F, Nishikawa H. Higher cerebrospinal fluid pH may contribute to the development of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. Transl Stroke Res. 2017;8:165–73.
5. Suzuki H, Kawakita F. Tenascin-C in aneurysmal subarachnoid hemorrhage: deleterious or protective? Neural Regen Res. 2016;11:230–1.
6. Fujimoto M, Shiba M, Kawakita F, Liu L, Shimojo N, Imanaka-Yoshida K, et al. Deficiency of tenascin-C and attenuation of blood-brain barrier disruption following experimental subarachnoid hemorrhage in mice. J Neurosurg. 2016;124:1693–702.
7. Liu L, Fujimoto M, Kawakita F, Nakano F, Imanaka-Yoshida K, Yoshida T, et al. Anti-vascular endothelial growth factor treatment suppresses early brain injury after subarachnoid hemorrhage in mice. Mol Neurobiol. 2016;53:4529–38.
8. Liu L, Kawakita F, Fujimoto M, Nakano F, Imanaka-Yoshida K, Yoshida T, et al. Role of periostin in early brain injury after subarachnoid hemorrhage in mice. Stroke. 2017;48:1108–11.
9. Fujimoto M, Shiba M, Kawakita F, Liu L, Shimojo N, Imanaka-Yoshida K, et al. Effects of tenascin-C knockout on cerebral vasospasm after experimental subarachnoid hemorrhage in mice. Mol Neurobiol. 2017;55:1951–8. https://doi.org/10.1007/s12035-017-0466-x.

10. Liu L, Fujimoto M, Nakano F, Nishikawa H, Okada T, Kawakita F, et al. Deficiency of tenascin-C alleviates neuronal apoptosis and neuroinflammation after experimental subarachnoid hemorrhage in mice. Mol Neurobiol. 2018; https://doi.org/10.1007/s12035-018-1006-z.
11. Suzuki H, Kanamaru K, Suzuki Y, Aimi Y, Matsubara N, Araki T, et al. Tenascin-C is induced in cerebral vasospasm after subarachnoid hemorrhage in rats and humans: a pilot study. Neurol Res. 2010;32:179–84.
12. Fujinaga K, Onoda K, Yamamoto K, Imanaka-Yoshida K, Takao M, Shimano T, et al. Locally applied cilostazol suppresses neointimal hyperplasia by inhibiting tenascin-C synthesis and smooth muscle cell proliferation in free artery grafts. J Thorac Cardiovasc Surg. 2004;128:357–63.
13. Nishino A, Umegaki M, Fujinaka T, Yoshimine T. Cilostazol attenuates cerebral vasospasm after experimental subarachnoid hemorrhage. Neurol Res. 2010;32:873–8.
14. Ito H, Fukunaga M, Suzuki H, Miyakoda G, Ishikawa M, Yabuuchi Y, et al. Effect of cilostazol on delayed cerebral vasospasm after subarachnoid hemorrhage in rats: evaluation using black blood magnetic resonance imaging. Neurobiol Dis. 2008;32:157–61.
15. Boulouis G, Labeyrie MA, Raymond J, Rodriguez-Régent C, Lukaszewicz AC, Bresson D, et al. Treatment of cerebral vasospasm following aneurysmal subarachnoid haemorrhage: a systematic review and meta-analysis. Eur Radiol. 2017;27:3333–42.
16. Senbokuya N, Kinouchi H, Kanemaru K, Ohashi Y, Fukamachi A, Yagi S, et al. Effects of cilostazol on cerebral vasospasm after aneurysmal subarachnoid hemorrhage: a multicenter prospective, randomized, open-label blinded end point trial. J Neurosurg. 2013;118:121–30.
17. Matsuda N, Naraoka M, Ohkuma H, Shimamura N, Ito K, Asano K, et al. Effect of cilostazol on cerebral vasospasm and outcome in patients with aneurysmal subarachnoid hemorrhage: a randomized, double-blind, placebo-controlled trial. Cerebrovasc Dis. 2016;42:97–105.
18. Suzuki S, Sayama T, Nakamura T, Nishimura H, Ohta M, Inoue T, et al. Cilostazol improves outcome after subarachnoid hemorrhage: a preliminary report. Cerebrovasc Dis. 2011;32:89–93.
19. Konczalla J, Seifert V, Beck J, Güresir E, Vatter H, Raabe A, et al. Outcome after Hunt and Hess grade V subarachnoid hemorrhage: a comparison of pre-coiling era (1980-1995) versus post-ISAT era (2005-2014). J Neurosurg. 2018;128:100–10.
20. Yoo AR, Koh SH, Cho GW, Kim SH. Inhibitory effects of cilostazol on proliferation of vascular smooth muscle cells (VSMCs) through suppression of the ERK1/2 pathway. J Atheroscler Thromb. 2010;17:1009–18.
21. Niki T, Mori H. Phase I study of cilostazol: safety evaluation at increasing single doses in healthy volunteers. Arzneimittelforschung. 1985;35(7A):1173–85.
22. Drake CG. Report of World Federation of Neurological Surgeons Committee on a universal subarachnoid hemorrhage grading scale [letter]. J Neurosurg. 1988;68:985–6.
23. Suzuki H, Muramatsu M, Tanaka K, Fujiwara H, Kojima T, Taki W. Cerebrospinal fluid ferritin in chronic hydrocephalus after aneurysmal subarachnoid hemorrhage. J Neurol. 2006;253:1170–6.
24. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol. 1995;57:289–300.
25. Austin PC. Optimal caliper widths for propensity-score matching when estimating differences in means and differences in proportions in observational studies. Pharm Stat. 2011;10:150–61.
26. Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med. 2009;28:3083–107.
27. Frontera JA, Claassen J, Schmidt JM, Wartenberg KE, Temes R, Connolly ES Jr, et al. Prediction of symptomatic vasospasm after subarachnoid hemorrhage: the modified fisher scale. Neurosurgery. 2006;59:21–7.
28. Yamaguchi-Okada M, Nishizawa S, Mizutani A, Namba H. Multifaceted effects of selective inhibitor of phosphodiesterase III, cilostazol, for cerebral vasospasm after subarachnoid hemorrhage in a dog model. Cerebrovasc Dis. 2009;28:135–42.
29. Uchida H, Mishima Y, Tanabe T, Furukawa K, Ohashi S, Sakaguchi S, et al. Therapeutic effect of OPC-13013 on chronic arterial occlusive diseases. Cardioangiology. 1985;17:421–32.
30. Nakatsuka Y, Kawakita F, Yasuda R, Umeda Y, Toma N, Sakaida H, et al. Preventive effects of cilostazol against the development of shunt-dependent hydrocephalus after subarachnoid hemorrhage. J Neurosurg. 2017;127:319–26.
31. Ahn SH, Savarraj JP, Pervez M, Jones W, Park J, Jeon SB, et al. The subarachnoid hemorrhage early brain edema score predicts delayed cerebral ischemia and clinical outcomes. Neurosurgery. 2017;83:137–45. https://doi.org/10.1093/neuros/nyx364.
32. Fujii M, Yan J, Rolland WB, Soejima Y, Caner B, Zhang JH. Early brain injury, an evolving frontier in subarachnoid hemorrhage research. Transl Stroke Res. 2013;4:432–46.
33. Suzuki H, Kanamaru K, Shiba M, Fujimoto M, Imanaka-Yoshida K, Yoshida T, et al. Cerebrospinal fluid tenascin-C in cerebral vasospasm after aneurysmal subarachnoid hemorrhage. J Neurosurg Anesthesiol. 2011;23:310–7.
34. Suzuki H, Nishikawa H, Kawakita F. Matricellular proteins as possible biomarkers for early brain injury after aneurysmal subarachnoid hemorrhage. Neural Regen Res. 2018;13:1175–8.
35. Okada T, Suzuki H. Toll-like receptor 4 as a possible therapeutic target for delayed brain injuries after aneurysmal subarachnoid hemorrhage. Neural Regen Res. 2017;12:193–6.
36. Al-Mufti F, Amuluru K, Smith B, Damodara N, El-Ghanem M, Singh IP, et al. Emerging markers of early brain injury and delayed cerebral ischemia in aneurysmal subarachnoid hemorrhage. World Neurosurg. 2017;107:148–59.
37. Zheng YK, Dong XQ, Du Q, Wang H, Yang DB, Zhu Q, et al. Comparison of plasma copeptin and multiple biomarkers for assessing prognosis of patients with aneurysmal subarachnoid hemorrhage. Clin Chim Acta. 2017;475:64–9.
38. Satoh S, Ikegaki I, Kawasaki K, Asano T, Shibuya M. Pleiotropic effects of the rho-kinase inhibitor fasudil after subarachnoid hemorrhage: a review of preclinical and clinical studies. Curr Vasc Pharmacol. 2014;12:758–65.
39. Chapados R, Abe K, Ihida-Stansbury K, McKean D, Gates AT, Kern M, et al. ROCK controls matrix synthesis in vascular smooth muscle cells: coupling vasoconstriction to vascular remodeling. Circ Res. 2006;99:837–44.