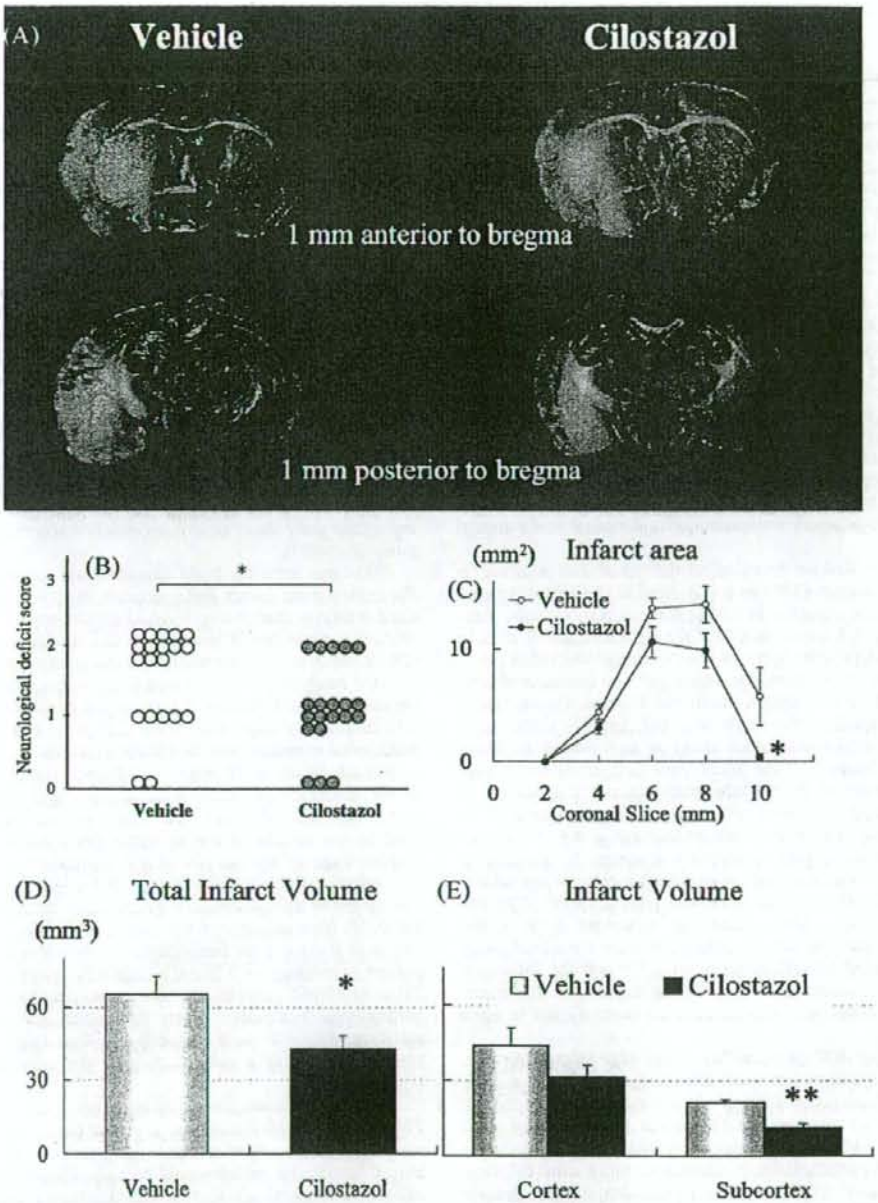

Fig. 1. Effect of cilostazol on parameters related to acute injury at 22 h after a 2-h focal middle cerebral artery (MCA) occlusion in mice. (A) 2,3,5-triphenyltetrazolium chloride (TTC)-stained coronal sections of brains from mice treated with vehicle (left) or cilostazol (right), location ±1 mm from bregma, showing infarct tissues (pale unstained region). (B) neurological deficit scores at 22 h after reperfusion. $^{*}P < 0.05$ versus vehicle (Mann–Whitney *U*-test, $n = 20$ indicating mice that were assessed for hemorrhagic transformation). (C) total infarct area, (D) total infarct volume, and (E) infarct volumes in cortex and subcortex. $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle (Student's *t*-test, $n = 10$). Data are expressed as means ± S.E.M.

A few years ago, Lee et al. reported that cilostazol prevented an increase in BBB permeability in rat brains subjected to MCA occlusion and reperfusion [10]. In the present study, we found that cilostazol significantly reduced the extent of Evans blue extravasation in mice subjected to a similar insult, supporting the idea that cilostazol limits or prevents BBB disruption after ischemia/reperfusion injury. However, the cerebral water content was not different between our two groups of mice, possibly because of the short time (2 h) between reperfusion and sampling in our experiment. Clinically, hemorrhagic infarction sometimes

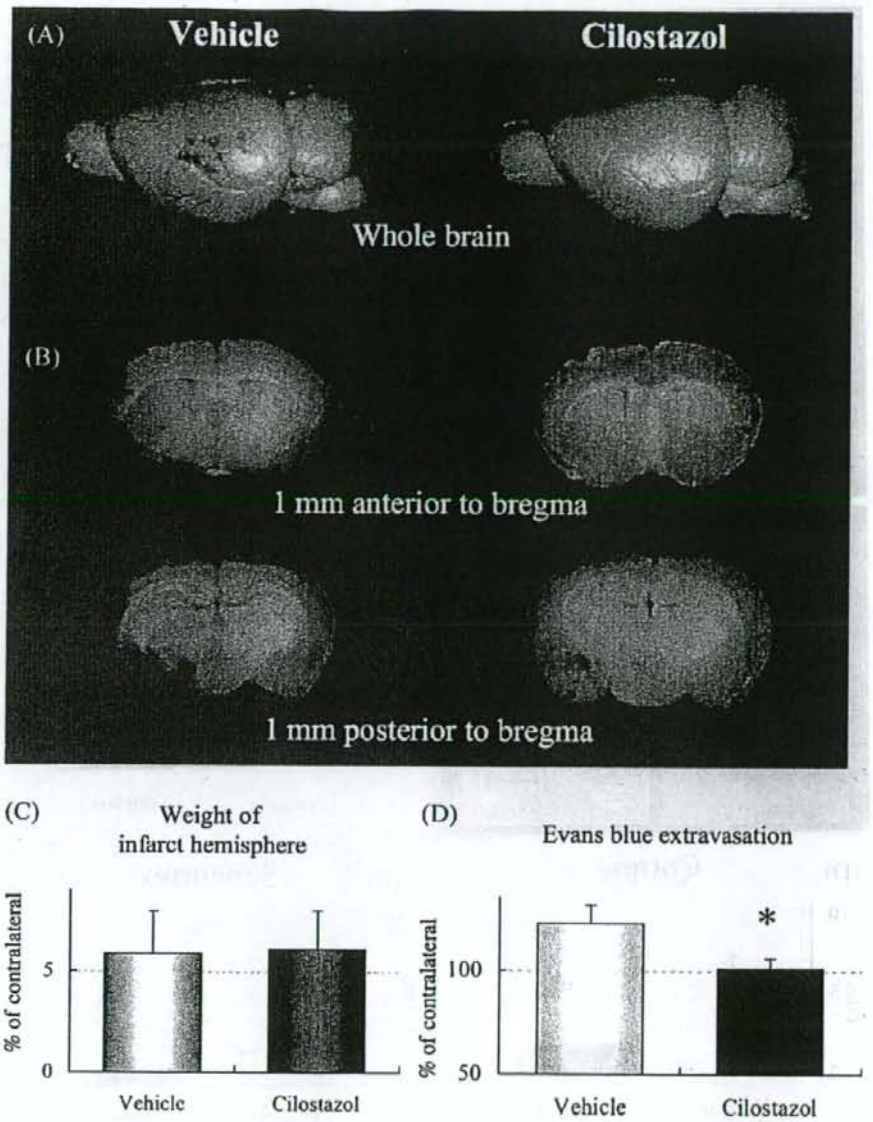

Fig. 2. Effect of cilostazol on blood–brain-barrier permeability, assessed using Evans blue extravasation, at 2 h after a 2-h focal middle cerebral artery (MCA) occlusion in mice. (A) Whole brains of mice treated with vehicle (left) or cilostazol (right) showing Evans blue extravasation in the dorsolateral cortex (pale stained region). (B) coronal sections of brains from mice treated with vehicle (left) or cilostazol (right), location ±1 mm from bregma, showing Evans blue extravasation in infarct area. (C) weight of infarct hemisphere (% of contralateral; $P = 0.94$, cilostazol versus vehicle). (D) quantitative analysis of Evans blue extravasation in ischemic hemisphere (% of contralateral). *$P < 0.05$ versus vehicle (Student's t-test, $n = 13$). Data are expressed as means ± S.E.M.

occurs after recanalization has been achieved by the application of thrombolytic therapy for acute ischemic stroke, and this clinically encountered phenomenon is similar to the hemorrhagic transformation detected in our transient ischemia model.

A method for the evaluation of hemorrhagic infarctions was previously reported by Aronowski et al. [1], and they recognized four subtypes of hemorrhagic transformation. Our model did not exhibit the parenchymal hematomas described in their report, a difference that might be related to the different methods employed for inducing ischemia. We evaluated the hemorrhagic transformation by a novel method (counting hemorrhagic points), a method that although very simple and objective, is semiquantitative.

The count of hemorrhagic spots and the Evans blue content in the cilostazol group were smaller by around 64% and 96%, respectively, than those in the vehicle group, whereas the corresponding value for infarct volume was approximately 35%. This reduction

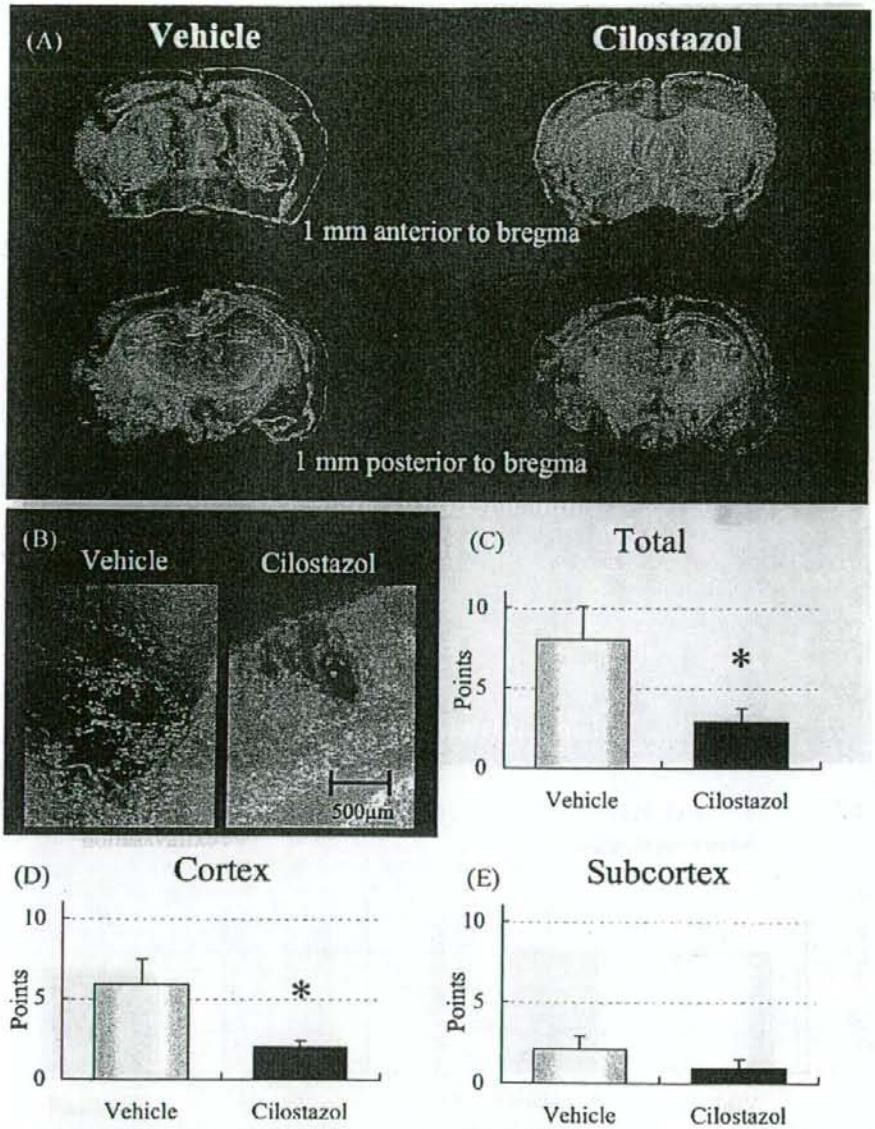

Fig. 3. Effect of cilostazol on hemorrhagic transformation at 22 h after a 2-h focal middle cerebral artery (MCA) occlusion in mice. (A) coronal sections of brains from mice treated with vehicle (left) or cilostazol (right) with no staining (location ±1 mm from bregma) showing hemorrhagic spots in the infarct area. Semiquantitative analysis of hemorrhagic transformation by counting the number of hemorrhagic spots >500 μm in diameter (one spot counting as one point). (B) Higher magnifications of hemorrhagic lesion area in vehicle (left, four points) and cilostazol group (right, one point). Bar = 500 μm. The average points in total slice, cortex, and subcortex ((C)–(E), respectively). *$P < 0.05$ versus vehicle (Mann–Whitney U-test, $n = 10$). Data are expressed as mean ± S.E.M.

in infarct volume with cilostazol was thus much smaller than the reduction in hemorrhagic transformation and BBB disruption. This suggests that the reduction in hemorrhagic transformation occurring after cilostazol treatment may not merely be secondary to this smaller brain infarct, but may also be due to a potent protection by cilostazol against hemorrhagic transformation. The reduction of Evans blue extravasation that occurs after brain ischemia in the cilostazol-treated group might be caused by decrease of hemorrhagic transformation. Clinically, it is desirable to protect against hemorrhagic infarction, which can result in a fatal prognosis, but as yet there is no specific provision for such protection.

Antiplatelet therapy may be beneficial for patients with acute ischemic stroke [4]. However, antiplatelet drugs have the side effect of hemorrhagic complications, and this side effect can cause patient deterioration during thrombolytic therapy for acute ischemic brain attack. The present findings indicate that cilostazol may be a drug

with the potential to reduce hemorrhagic complications. However, further experiments will be needed to confirm this, and to clarify the detailed mechanism(s).

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