Effects of a Selective Inhibitor of Cyclic AMP Phosphodiesterase on the Pial Microcirculation in Feline Cerebral Ischemia

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We evaluated the effects of cilostazol, a selective inhibitor of cyclic adenosine monophosphate phosphodiesterase, on the pial vessels of adult cats subjected to endothelial damage followed by middle cerebral artery occlusion. Six cats were treated with cilostazol and four with 30% N,N-dimethylformamide in 70% saline (solvent). The brain surface was irradiated with ultraviolet rays through a cranial window for 3 minutes to selectively damage the endothelium of the pial vessels in both groups. Beginning 32 minutes after termination of the irradiation, the middle cerebral artery was occluded for 30 minutes. Thirty minutes before occlusion, intravenous infusion of 30 µg/kg/min cilostazol or 0.1 ml/kg/min solvent was begun and continued until the end of the study. Before occlusion, the infusion of cilostazol induced a significant (p<0.05) dilatation while the infusion of solvent produced no significant changes in the diameter of the pial arteries. The pial veins of solvent-treated cats showed significant (p<0.05) constriction during occlusion, whereas cilostazol-treated cats exhibited only mild constriction of the pial veins. The formation of platelet thrombi after occlusion was significantly (p<0.05) inhibited in the pial veins of cilostazol-treated compared with solvent-treated cats. Similarly, the microcirculation of the pial veins was effectively restored after reopening of the middle cerebral artery in cilostazol-treated compared with solvent-treated cats. Our data suggest that cilostazol is an effective antithrombotic agent as well as a potent vasodilator acting on vascular smooth muscle. (Stroke 1989;20:668-673)

It is widely accepted that calcium ions (Ca^{2+}), mainly from an intracellular source, play a key role in platelet activation such as in shape change, aggregation, and the secretion response. Measurement with fluorescent Ca^{2+} indicators has demonstrated that a rapid rise in intracellular Ca^{2+} concentration accompanies platelet activation. Recent studies have suggested that such a rise can be inhibited by cyclic adenosine monophosphate (cAMP), which appeared to act primarily through inhibition of Ca^{2+} mobilization and/or through enhanced sequestration of Ca^{2+}. Cyclic nucleotide phosphodiesterase catalyzes the hydrolysis of cAMP and cyclic guanosine monophosphate (GMP), and this is the only known catabolic mechanism for these regulatory nucleotides in platelets. Inhibition of phosphodiesterase is thus expected to increase the cAMP content in platelets, preventing platelet activation.

Cilostazol (OPC-13013), a selective inhibitor of cAMP phosphodiesterase, has been shown to prevent human platelet aggregation in vitro. Cilostazol has also been reported to relax vascular strips prepared from rabbit aorta. In view of the implied therapeutic potential for it in cerebral ischemia, we evaluated the effects of cilostazol on microcirculatory derangements following middle cerebral artery (MCA) occlusion (MCAO) in feline pial vessels with endothelial damage. The endothelium was selectively damaged by irradiation with ultraviolet (UV) rays, using a technique developed in our laboratory.

Materials and Methods

We used 10 adult cats of either sex weighing 2.2–3.6 kg. The anesthesia employed and subsequent respiratory control and placement of the cranial window were as described. Briefly, a cranial window made of quartz with a stainless steel frame was...
screwed into the left parietal region of the calvaria after placing the head of each cat in a head holder. Catheters were inserted into the right femoral artery and vein to record the systemic arterial blood pressure and to infuse cilostazol or solvent, respectively.

Our experimental setup was as described. Briefly, the pial vessels and platelets were visualized at ×200 magnification on a television (TV) monitor, and the diameters of the target vessels of interest were recorded continuously using a multipen recorder. The output of the TV monitor was recorded with a videotape recorder (VO-5850, Sony, Tokyo, Japan) so the images could be analyzed later with an image analyzer developed in our laboratory by replaying the tape. Platelet aggregates can be distinguished from the vessel wall and plasma by their optical properties using the image analyzer, which converts an analog video image into 512×512 digital data and generates an 8-bit (256-unit) gray value for each pixel. A xenon lamp (150 W) with a heat-cut filter and a specially designed glass (low-cut) filter that cuts wave lengths of <420 nm was used to illuminate the brain surface through a quartz glass fiber. UV irradiation of the pial vessels was carried out with the same system without the low-cut filter. The intensity of UV rays at the brain surface was irradiated with UV rays for 3 minutes to slightly damage the endothelium of the pial vessels. In six cats (Group 1), continuous intravenous infusion of cilostazol at 30 μg/kg/min was begun 2 minutes after termination of the UV irradiation and was maintained throughout the remainder of the study; the cilostazol was dissolved in a solution of 30% N,N-dimethylformamide and 70% saline. Thirty minutes after the start of cilostazol infusion, the left MCA was occluded with a Scoville clip for 30 minutes by the transorbital approach. The MCA was then reopened, and observation continued for 30 more minutes. In four cats (Group 2), continuous intravenous infusion of the solvent at 0.1 ml/kg/min was begun 2 minutes after termination of the UV irradiation and was maintained throughout the remainder of the study; the protocol was identical to that in Group 1.

Microcirculatory derangements derived from activated platelets were assessed by the incidence of three phenomena: 1) formation of platelet thrombi (adhesion of platelet aggregates to the endothelium) after MCAO, 2) total blockade of blood flow by enlarging thrombi in the vessels after MCAO, and 3) failure of complete recovery of the microcirculation following reopening of the MCA.

Table 1 shows the control MABP and diameter of the observed pial arteries and veins in both groups. There was no significant difference between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>Mean arterial blood pressure (mm Hg)</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pial artery</td>
</tr>
<tr>
<td>1</td>
<td>Cilostazol</td>
<td>6</td>
<td>100.0±10.8</td>
<td>200.1±13.7</td>
</tr>
<tr>
<td>2</td>
<td>Solvent</td>
<td>4</td>
<td>100.5±8.2</td>
<td>217.0±52.5</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

Results

Table 1 shows the control MABP and diameter of the observed pial arteries and veins in both groups. There was no significant difference between groups.

Figure 1 illustrates sequential changes in the video images of pial vessels in Group 1. Before MCAO, 30-minute infusion of cilostazol induced marked dilatation of a large pial artery (center of the image) and its branch compared with the control image. Ten minutes after MCAO, the diameter of the pial artery was definitely reduced compared with that before MCAO but was still larger than control; no platelet thrombus was observed in either the pial artery or the pial vein (left side of the image). After reopening of the MCA, the pial artery and vein underwent significant dilatation, and blood flow was completely restored.

Figure 2 illustrates sequential changes in the video images of pial vessels in Group 2. Before MCAO, 30-minute infusion of solvent induced slight dilatation of both a large pial artery (left side of the image) and a vein (right side of the image). Ten minutes after MCAO, the pial artery exhibited marked constriction and numerous platelet aggregates began to adhere to the left side of the vessel wall; in the pial vein, a developing platelet thrombus, appearing as a white plaque in the lumen, blocked blood flow completely. After reopening of the MCA, a large platelet thrombus still remained in the vein, although it was partially disintegrated, and blood flow was resumed to a very limited extent; the pial artery underwent marked dilatation, with residual platelet thrombi remaining on the vessel wall.

The top and bottom panels in Figure 3 show the percent changes in pial artery diameter and MABP, respectively. UV irradiation immediately caused moderate dilatation of the pial arteries (11.7–18.6% of control diameter) in both groups, which continued until the start of infusion; the degree of dilatation appeared to be greater in Group 1 than in Group 2, possibly due to the smaller control diameter in Group 1.

After the initiation of cilostazol infusion, the pial arteries showed significant dilatation compared with
FIGURE 1. Sequential changes in video images of pial vessels in cilostazol-treated cats before and after middle cerebral artery occlusion (MCAO). Note significant dilatation 30 minutes after initiation of cilostazol infusion (before MCAO) and no thrombus formation after MCAO. Bar=100 μm.

the irradiation diameter (fifth minute of the study). After 10 minutes of cilostazol infusion, pial artery diameter reached a maximum (36.6±9.0% of control diameter) and then gradually declined with decreasing MABP. Five and ten minutes after MCAO, the arteries revealed significant constriction compared with the infusion diameter (35th minute of the study), but thereafter a gradual recovery of the diameter was noted. Following reopening of the MCA, in spite of a significant decrease in MABP, the arteries exhibited a marked dilatation that was significantly different from the irradiation diameter.

In Group 2, solvent infusion caused a slight dilatation accompanied by a slight increase in MABP. After MCAO, the artery diameter revealed a pattern of changes similar to that in Group 1 but did not show significant dilatation after reopening of the MCA.

FIGURE 2. Sequential changes in video images of pial vessels in solvent-treated cats before and after middle cerebral artery occlusion (MCAO). Platelet thrombus developed in vein after MCAO and remained after reopening of MCA. Bar=100 μm.
The percent changes in pial vein diameter are illustrated in the middle panel of Figure 3. In contrast to the pial arteries, the veins did not exhibit definite dilatation after UV irradiation in either group. The infusion of cilostazol or solvent induced almost no changes in vein diameter before MCAO. Following MCAO, Group 2 revealed significant constriction (19.3–31.5% of control diameter), whereas Group 1 showed only mild constriction. After reopening of the MCA, both groups demonstrated moderate dilatation, but recovery of the control diameter was slightly more pronounced in Group 1 than in Group 2.

Table 2 summarizes the hemorheologic features in the pial vessels of both groups. Platelet thrombus formation was noted after MCAO in the pial artery in only one of the six cats in Group 1 (16.7%) but in three of the four cats in Group 2 (75.0%); in the pial vein, Group 1 exhibited a significantly lower incidence (16.7%) than Group 2 (100%) (p<0.05, Fisher's exact test). There was no significant difference in the incidence of stasis of blood after MCAO between groups in either the arteries or the veins. Similar incidences of complete recovery of blood flow following reopening of the MCA was observed in the pial arteries of both groups. In the pial veins, however, complete recovery was observed in five of the six cats in Group 1 (83.3%) but in none of the four cats in Group 2 (p<0.05, Fisher's exact test).

Discussion

Our study demonstrates that administration of cilostazol significantly dilated pial arteries of cats. In addition, cilostazol effectively prevented platelet thrombus formation in pial veins after MCAO.

Table 2. Hemorheologic Features of Pial Vessels in 10 Cats After Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>Complete recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Artery (vein)</td>
</tr>
<tr>
<td>1</td>
<td>Cilostazol</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>1*</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Solvent</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are number of cats.
*p<0.05 incidence different from Group 2, Fisher's exact test.
ensuring good recovery of blood flow following reopening of the MCA. Cilostazol has been reported to inhibit selectively cAMP phosphodiesterase in vascular smooth muscle as well as in platelets.\textsuperscript{10,12} Since cyclic nucleotide phosphodiesterase is the only known pathway for degradation of cyclic nucleotide,\textsuperscript{10} we expected the concentration of cAMP to rise in such tissues during the infusion of cilostazol. It is well known that cilostazol is one of the prominent second messengers controlling various important cellular functions.\textsuperscript{15}

Multiple steps in the excitation–contraction process have been postulated to represent the primary locus for cAMP-dependent relaxation of smooth muscle.\textsuperscript{16,17} cAMP-dependent protein kinase alone or with cAMP is suggested to stimulate the uptake of sarcoplasmic Ca\textsuperscript{2+} by microsomal vesicles,\textsuperscript{18} decreasing the sarcoplasmic Ca\textsuperscript{2+} concentration. Other investigators have emphasized cAMP-dependent regulation of transmembranous ion transport such as increased active transport of Ca\textsuperscript{2+} from the cytoplasm to the extracellular space triggered by cAMP.\textsuperscript{19} The phosphodiesterase activity in veins is generally much lower than that in arteries.\textsuperscript{16} Such a distribution pattern of phosphodiesterase appears to be consistent with our results showing selective dilatation of pial arteries without definite changes in the diameter of pial veins during infusion of cilostazol. After the start of cilostazol infusion, MABP gradually declined, suggesting that this agent dilated not only pial arteries but also systemic arteries. We have observed complete disappearance of the autoregulatory response of pial arteries with endothelial damage produced by UV irradiation.\textsuperscript{20,21} Once exposed to UV irradiation, pial arteries constricted in parallel with an MABP decrease under normal conditions. Therefore, it seems reasonable to speculate that cilostazol may preferentially dilate pial arteries and not systemic arteries. In fact, cilostazol has been shown to increase cerebral blood flow in a dose-dependent fashion in cats.\textsuperscript{22} Our study also suggests that the dilatation of pial vessels induced by cilostazol does not require the presence of intact endothelium.

A rise in the cytoplasmic free Ca\textsuperscript{2+} concentration has been regarded as the final common pathway for the cytoskeletal rearrangement of platelet shape, secretion of granular contents, and aggregation.\textsuperscript{1,2,4,8} cAMP has long been considered to be involved in the inhibition of platelet aggregation produced by prostaglandins E and I\textsubscript{2}, isoprenaline, and adenosine.\textsuperscript{23-24} Recent studies using the fluorescent Ca\textsuperscript{2+} indicator quin2 have provided direct evidence that cAMP inhibits a rise of the free Ca\textsuperscript{2+} concentration in platelets exposed to aggregating agents such as thrombin.\textsuperscript{7,8} CAMP is also suggested to stimulate the resequestration of Ca\textsuperscript{2+} that has already been released into the cytoplasm by thrombin.\textsuperscript{6,8}

Kimura et al\textsuperscript{11} have reported that cilostazol potently and dose-dependently inhibits platelet aggregation induced by adenosine diphosphate or collagen in vitro or in vivo. They also found that cilostazol inhibits human platelet aggregation to a much greater extent than does acetylsalicylic acid.\textsuperscript{11} Further, cilostazol has a dispersing effect on platelet aggregation, as does prostaglandin I\textsubscript{2}.\textsuperscript{11} In line with these pharmacologic properties, we found that cilostazol effectively prevents the formation of platelet thrombi in pial veins after MCAO; in pial arteries thrombus formation was similarly inhibited, but statistical significance was not reached. These antithrombotic effects of cilostazol in conjunction with its potent vasodilative action apparently lead to complete recovery of blood flow following reopening of the MCA.

Pial veins in Group 2 cats exhibited significant constriction after MCAO. This phenomenon may be explained by two mechanisms: the impairment of the autoregulatory response of pial vessels due to endothelial damage\textsuperscript{20,21} and the release of vasoconstrictive substances from aggregating platelets.\textsuperscript{22} In contrast, the constriction of pial veins in Group 1 cats was not significant after MCAO, suggesting that inhibition of platelet aggregation plus the vasodilative action of cilostazol may prevent the vasoconstriction seen in Group 2.

It has been shown that UV irradiation causes dilatation of the systemic vessels in vitro,\textsuperscript{26} and a recent study has indicated that an increase of cyclic GMP content in vascular smooth muscle accompanies such dilatation.\textsuperscript{27} We found that pial vessels, especially arteries, dilated rapidly in vivo under the influence of UV irradiation. Cilostazol produced significant dilatation of pial arteries that had already dilated after UV irradiation, indicating that both cAMP and cyclic GMP may be intimately involved in the regulation of vascular smooth muscle function. Further detailed studies are needed to elucidate the control mechanism of the vasodilatation tightly coupled with the metabolism of each cyclic nucleotide.

In conclusion, we have demonstrated that selective inhibition of cAMP phosphodiesterase by cilostazol exerted beneficial effects on the pial microcirculation in cerebral ischemia, providing an adequate rationale for the currently ongoing clinical trials of this agent.

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