た細胞とGPIb alpha-PCDNA3.1hygro(+)導入のものでは全体の細胞数に違いは認められなかった。GPIb alphaのsiRNAを導入した検討の結果、巨核球を示唆するゲートに入った細胞はnegative control siRNAを導入した細胞では20.3%であり、GPIb alpha-siRNA1を導入したものでは9.8%、GPIb alpha-siRNA2を導入したものでは18.8%であった。血小板を示唆するゲートに入った細胞はnegative control siRNAを導入した細胞では2.9%であり、GPIb alpha-siRNA1を導入したものでは1.6%、GPIb alpha-siRNA2を導入したものでは5.3%であった。FITCのmeanレベルは巨核球、血小板ともにnegative control siRNA、GPIb alpha-siRNA1、GPIb alpha-siRNA2を導入した細胞において違いは認めなかった。これら結果はGPIb alpha-siRNA1はこのノックダウンに有用と考えられるがGPIb alpha-siRNA2はその有用性に乏しい、と考えられる。また、GPIb alpha-siRNA1は細胞に導入されており、その結果巨核球分化と血小板産生が抑制されたことを示唆している。この導入により巨核球、血小板ひとつあたりのGPIb alpha発現量は変化していないと考えられる。

(2) 超微細構造像による巨核球分化と血小板産生の研究：OP9培養システムにおいてES細胞から、Serum-free liquid culture システムにおいてヒトCD34陽性細胞から、巨核球・血小板へ*in vitro*分化誘導を行った。形態観察、フローサイトメトリーによるCD41陽性細胞の検出の結果から培養8日で未成熟巨核球、培養12日で成熟巨核球、そして培養15日で血小板産生が示唆された。マウスES細胞およびヒトCD34陽性細胞をそれぞれ巨核球・血小板に*in vitro* 分化誘導を行い、その培養5日目、8日目、12日目、15日目の細胞を電子顕微鏡観察、免疫電子顕微鏡観察した。各過程の超微細構造像を電子顕微鏡・免疫電顕観察により検討した結果、培養8日、12日で顆粒や分画膜を有する巨核球様細胞を認めたが, それら細胞辺縁の形態を2種類(proplatelet様と平滑辺縁)認めた。proplatelet様辺縁巨核球はfibrinogenを除く(血小板中のFibrinogenは血漿から取り込まれているものとされている)各種抗体と反応したが平滑辺縁巨核球様の細胞はいずれの抗体にも反応しなかった。培養12日、15日で巨核球細胞質の四散, いわゆるglobal fragmentationを認めた。電子顕微鏡・免疫電顕観察により巨核球分化、血小板産生に誘導されている細胞ではアポトーシスが観察された。培養8日目の細胞、未

成熟の巨核球でまだ顆粒は少なく、巨核球に特有のdemarcation membraneの出来始めを認めた。核の観察ではtypicalなearly apoptosisを示すchoromatinのcondensationを認めた。培養12日目の細胞、成熟巨核球では顆粒の存在がはっきりと数多く見え、
apoptosis に関しては nuclear material の condensation が多くなり apoptotic body を認めた。この培養 12 日目の細胞は培養 8 日の細胞よりも apoptosis は進んでいると考えられた。

D. 考察

(1) マウス ES 細胞、ヒト造血幹細胞を用いた遺伝子ターゲティング巨核球・血小板産生システムのプロトコールの確立: 本研究では抗血小板薬の反応性に関連する遺伝子の同定のために検診受診者(抗血小板薬非服用者)や抗血小板薬服用者の血液サンプルを用いた血小板機能検査や遺伝子解析（網羅的解析および候補因子アプローチ)を行っている。これら検討により得られた結果は、遺伝子改変の実験検討による検証が必要とされるが血小板は無核であるため、遺伝子改変ができない。これは血小板研究の主要な問題点とされている。そこで本研究では幹細胞に対して遺伝子改変を行い、それを *in vitro* で分化誘導を行うことにより、遺伝子改変された巨核球・血小板を得る実験システム樹立することに着手した。マウス ES 細胞は強い増殖能を有するため、実験プロトコールの検討に適している。一方、ヒト造血幹細胞を個体から得るためにはドナーの負担が大きいため、再現性が要求される実験には適していない。したがって平成 17 年度までは、マウス ES 細胞を用いた基礎検討を行ってきた。本年度はこれまでの成果から、ヒト造血幹細胞を用いた実験システムの構築を行った。ヒト造血幹細胞を 4 日間 Serum-free liquid culture システムで培養後、エレクトロポレーション法により遺伝子導入を行うプロトコールを作成した。遺伝子導入効率の条件検討の結果から、ヒト造血幹細胞を 4 日間 Serum-free liquid culture システムで培養後の細胞を導入に用いているが内在している因子の干渉を受けることが考えられる。Preliminary な実験で内在する GPIb alpha とは異なる遺伝子多型の配列を有する発現ベクターを導入した結果、RNA の多くは外来遺伝子の多型配列を示していた。しかし内在している因子の干渉を受ける可能性を出来る限り少なくするための条件検討は今後も続ける必要がある。

(2) 超微細構造像による巨核球分化と血小板産生の研究: アスピリンは血小板のみならず、巨核球に対しても有用性があることが報告されている。我々の平成17年度の研究においても、マウスES細胞、アスピリン存在下での *in vitro* 分化誘導により得られた巨核球と血小板はそれぞれ血小板活性化の刺激に対して抑制作用を示した。アスピリンは細胞アポトーシスに関与していることが報告されているが「巨核球のアポトーシスにアスピリンが関与しているかどうか?」は不明であることに加え、「巨核球分化や巨核球からの血小板分離にアポトーシスが関与しているかどうか?」についても議論中である。巨核球からの血小板分離にはproplatelet theoryやexplosive fragmentation theoryが提唱されているが未だ議論が分かれ、その詳細は十分に解明されていない。今回の結果は巨核球分化、血小板産生にアポトーシスが関与していることを形態観察により認めた。さらに幹細胞から血小板産生までを経時的に検討できるシステムの利点を活かし、巨核球からの血小板分離の機序を研究した。その結果、巨核球からの血小板分離における中期から後期はglobal fragmentationの関与が示唆された。

E. 結論

抗血小板薬の反応性と関連する因子の研究として、マウスES細胞・ヒト造血幹細胞から *in vitro* 分化誘導による巨核球分化・血小板産生システムを用いた抗血小板薬の反応性と関連する因子の基礎検討を行った。ヒト造血幹細胞を用いた際の遺伝子改変巨核球・血小板を得るプロトコールを確立したことを認めた。さらに今回、造血幹細胞から血小板産生の各過程を電子顕微鏡観察ならびに免疫電子顕微鏡観察により検討した結果、血小板産生にアポトーシスが関与すること、血小板産生時に巨核球のglobal fragmentationが起きることを認めた。

F. 健康危険情報

現段階では上記の結果は実際の臨床の現場で疾病予防・治療に還元できるものではない。今後の更なる検討が必要と考えられる。

G. 研究発表

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H. 知的所有権の取得

特許取得　なし
実用新案登録　なし
その他　なし

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研究成果の刊行物・別冊

CHAPTER 64

Cilostazol

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I. Introduction

Platelet adhesion to blood vessel walls and aggregation are crucial physiological events in thrombosis and hemostasis. Excessive platelet accumulation at sites of atherosclerotic plaque rupture causes occlusion of blood vessels and leads to ischemia. In addition, platelet aggregates release substances acting on vascular tissues such as the platelet-derived growth factor (PDGF) that induce intimal hyperplasia. These phenomena are responsible for cardiovascular ischemic diseases such as acute coronary syndromes (see Chapter 35), peripheral arterial diseases (see Chapter 37), and stroke (see Chapter 36). In order to treat ischemic diseases resulting from platelet aggregation, many kinds of antiplatelet drugs are widely used in clinical situations.[1]

Cilostazol (Pletal) (Fig. 64-1) is an oral selective cyclic nucleotide phosphodiesterase 3 (PDE3) inhibitor with antiplatelet, vasodilatory, and antimitogenic effects.[2-5] Cilostazol has been clinically used for treatment of chronic peripheral arterial occlusion and stroke in 13 countries, including Japan, the United States, and the United Kingdom. Cilostazol was first approved in Japan in 1988 and has subsequently been approved in 12 other countries. In the United States, cilostazol has been clinically investigated since 1993 in patients with intermittent claudication, and it was approved by the Food and Drug Administration (FDA) in 1999 for this indication. This chapter reviews the pharmacology and clinical utility of cilostazol.

II. Mechanism of Action

It is well established that cyclic adenosine monophosphate (cAMP)-elevating agents, such as adenosine, prostaglandin I_2 (PGI_2), PGE_1, and PDE inhibitors, and cyclic guanosine 5′-monophosphate (cGMP)-elevating agents, such as nitric oxide (NO) donors, are able to inhibit platelet functions. Elevation of cAMP/cGMP concentrations can be accomplished either directly by stimulation of adenylate/guanylate cyclases or indirectly by inhibition of PDEs.[6] It has been considered that increased cAMP/cGMP in platelets activates cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG) and thus regulates platelet activation and aggregation responses by phosphorylating intracellular protein substrates, such as the IP3 receptor, phospholipase Cβ, glycoprotein (GP) Ibβ, $G\alpha_{13}$, RapIb, actin binding protein, vasodilator-stimulated phosphoprotein (VASP), and PDE3.[7] In most cells, including platelets, the intracellular effects of cAMP are primarily mediated by PKA and the protein substrates. However, the detailed signal transduction pathways remain unclear.

The intracellular levels of cAMP and cGMP are regulated by synthesizing systems, which include adenylate cyclase and guanylate cyclase, and hydrolytic systems, which include PDEs. In mammalian tissues, 11 isozymes of PDE (PDE1 through PDE11) have been identified according to their primary structures, substrate affinities, and inhibitor sensitivities (Table 64-1).[8,9] Most cell types express one or more PDE isozymes, which are expressed in tissue- and cell-specific distribution patterns, each regulating intracellular cAMP and/or cGMP levels in different cellular compartments and in different manners. The PDE activity of platelets has been reported to be mainly due to PDE3 and PDE5, with a minor activity for PDE2.[10,11] PDE2 and PDE3 preferentially hydrolyze cAMP, whereas PDE5 specifically hydrolyzes cGMP. It has been reported that the inhibition of PDE3 is important for the suppression of adenosine diphosphate (ADP)-induced platelet aggregation, whereas that of PDE5 is linked to a reduction of serotonin release.[12]

Cilostazol selectively inhibits PDE3 isozyme by a cAMP-competitive mechanism (Table 64-2).[5] Cilostazol potently inhibits the activity of PDE3A, a cardiovascular subtype of PDE3 (IC_{50} 0.20 μM), and increases intracellular cAMP concentrations and activates PKA in human platelets. Tyr751, Thr844, Asp950, Phe972, and Gln975 in the catalytic domain of PDE3A are key residues for the binding of

cilostazol.[13] Inhibitory effects of PDE3 inhibitors on PDE3A activity are highly correlated with their inhibition of platelet aggregation induced by thrombin, ADP, or collagen.[5] The pharmacological effect of cilostazol is therefore considered to be due to elevation of intracellular cAMP levels by inhibition of PDE3A activity in platelets.

Cilostazol induces the phosphorylation of VASP in platelets, mediated by PKA activation, despite the weak stimulatory effect on cAMP accumulation in comparison with forskolin, PGE_1, or PGI_2.[5,14] VASP is a 46 to 50 kDa protein, which was found concentrated along highly dynamic filamentous membrane structures, in focal adhesions and cell–cell contacts.[7,15] VASP modulates actin polymerization and actin filament bundling and integrin activation. VASP is phosphorylated in human platelets at Ser157, Ser239, and Thr278 with different affinities by both PKA and PKG. VASP phosphorylation downregulates its interaction with actin filaments.[16] VASP phosphorylation has been shown to closely correlate with the inhibition of fibrinogen binding to integrin αIIbβ3 and the inhibition of platelet aggregation and adhesion.[17,18] The VASP phosphorylation assay is also useful for quantifying the antiplatelet effect of clopidogrel.[19] NO donors induce VASP phosphorylation with a marked increase in cGMP levels, without influencing cAMP. The fact that VASP phosphorylation in response to various plate-

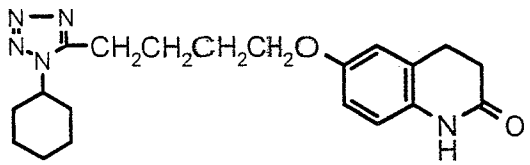

Figure 64-1. Chemical structure of cilostazol: 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone.

Table 64-1: PDE Isozyme Family

Isozyme	Affinity, K_m (μM) cAMP	cGMP	Subtype	Major Tissue Distribution
PDE1 (Ca^{2+}/CaM-dependent)	1–12	1–3	1A, 1B, 1C	Brain, heart, SMC, lung
PDE2 (cGMP-stimulated)	30	15	2A	Adrenal, heart, brain, kidney, liver, platelet
PDE3 (cGMP-inhibited)	0.1-0.4	0.03-0.3	3A, 3B	Platelet, heart, SMC, adipocyte, liver, β cell
PDE4 (cAMP-specific)	1–3	>300	4A, 4B, 4C, 4D	Brain, leukocyte, testis, EC, SMC, heart
PDE5 (cGMP-specific)	>100	0.5-5	5A	Lung, platelet, SMC
PDE6 (photoreceptor cGMP-specific)	>100	5-20	α, β, γ	Retina
PDE7 (cAMP-specific, rolipram-insensitive)	0.2	—	7A, 7B	Skeletal muscle, T cell
PDE8 (cAMP-specific, IBMX-insensitive)	0.055	124	8A, 8B	Testis, thyroid, liver, kidney, ovary, brain
PDE9 (cGMP-specific, IBMX-insensitive)	230	0.07-0.17	9A	Small intestine, kidney, liver, lung, brain, heart
PDE10 (cAMP/cGMP-specific)	0.05	3	10A	Brain, thyroid, testis
PDE11 (cAMP/cGMP-specific)	3.3	5.7	11A	Prostate, testis, pituitary, skeletal muscle, liver

CaM, calmodulin; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; EC, endothelial cells; IBMX, isobutyl methyl xanthine; PDE, phosphodiesterase; SMC, smooth muscle cells.

Table 64-2: Inhibitory Effects of Cilostazol on PDE Isozymes

	IC_{50} (μM) PDE-1	PDE-2	PDE-3A	PDE-3B	PDE-4	PDE-5	PDE-7
Cilostazol	>100	45.2	0.20	0.38	88.0	4.4	21.4
Specific inhibitor	Vinpocetine, 23.2	EHNA, 9.2	Cilostamide, 0.027	Cilostamide, 0.075	Rolipram, 0.45	Dipyridamole, 0.26	

let inhibitors is known to be mediated by either PKA or PKG[7] suggests that a small increase in cAMP due to PDE3 inhibition is sufficient to activate PKA/PKG. The activity of PDE3 may be restricted to distinct compartments from PDE2 and PDE5 within platelets.

The inhibition of platelet function by cilostazol appears to be due to decreased intracellular Ca^{2+} levels resulting from increased cAMP levels.[5,20] However, cilostazol also inhibits calcium ionophore-induced platelet aggregation, so the detailed mechanism of action remains unclear. It is reported that the inhibition of agonist-evoked elevations in intracellular Ca^{2+} concentration by cAMP/cGMP-elevating agents might have two components — a PKA/PKG-dependent mechanism for the inhibition of Ca^{2+} release from intracellular stores and a PKA/PKG-independent mechanism for the direct inhibition of Ca^{2+} entry mediated by protein tyrosine phosphatases.[21] These results suggest that the inhibitory effect of cilostazol on platelet aggregation might be mediated by PKA/PKG-dependent mechanisms, such as VASP phosphorylation and the inhibition of Ca^{2+} release, and PKA/PKG-independent mechanisms.

III. Antiplatelet Effects

Cilostazol inhibits platelet aggregation induced by various agonists in humans, mice, rabbits, and dogs.[3] IC_{50} values of cilostazol range from 3.6 to 15.0 μM for human platelet aggregation induced by various agonists in platelet-rich plasma and whole blood (Table 64-3).[3,22] Unlike aspirin, which inhibits only secondary platelet aggregation, cilostazol, with its different mode of action, inhibits not only primary but also secondary platelet aggregation induced by ADP or epinephrine. It is known that platelet aggregation is also induced by the high shear stress generated by blood flow. This aggregation appears to play an important role in the pathogenesis of arterial thrombosis.[23] Cilostazol inhibits shear stress-induced platelet aggregation (SIPA), with an IC_{50} of 15.0 μM, whereas aspirin has no effect.[24] Cilostazol inhibits the collagen- or thrombin-induced binding to platelets of PAC-1 (a monoclonal antibody specific for the activated GPIIb-IIIa complex [integrin αIIbβ3]).[20] Therefore, it is suggested that cilostazol inhibits platelet aggregation by suppressing GPIIbIIIa activation (Fig. 64-2). Cilostazol

Table 64-3: Inhibitory Effects of Cilostazol on Platelet Aggregation in Human PRP

	IC_{50} (μM)							
	ADP	Collagen	Thrombin	Arachidonic Acid	Epinephrine	U46619[a]	A23187[b]	SIPA[c]
Cilostazol	12.8	3.9	4.9	3.6	11.5	15.0	14.7	15.0
Aspirin	>1000	148	>1000	98.6	83.0			NE

[a]Thromboxane A_2 analogue.
[b]Calcium ionophore.
[c]Shear stress-induced platelet aggregation. NE, no effect.

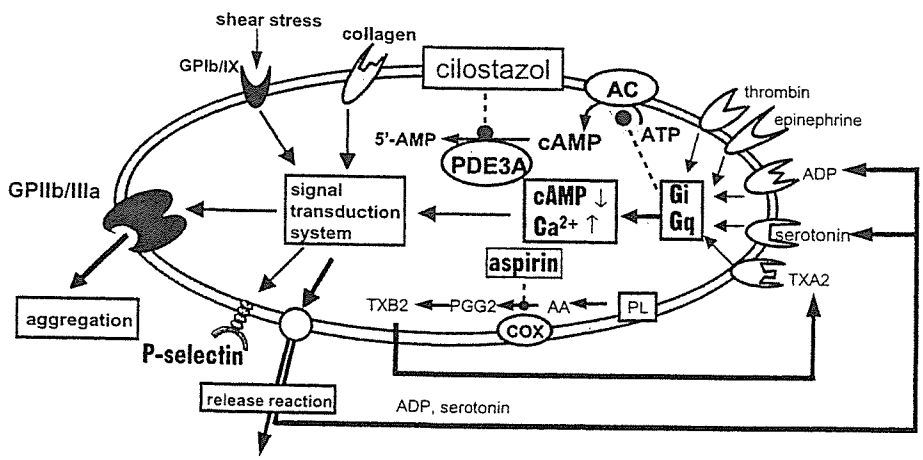

Figure 64-2. Inhibitory effects of cilostazol on agonist-induced platelet aggregation and release reaction. Cilostazol inhibits cyclic adenosine monophosphate (cAMP) hydrolysis via PDE3A inhibition and increases cAMP concentrations. As a result, cAMP inhibits platelet aggregation and the release reaction (including PDGF, P-selectin, and TXB_2 production). AA, arachidonic acid; AC, adenylate cyclase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; COX, cyclooxygenase; PDE, phosphodiesterase; PG, prostaglandin; PL, phospholipid; TX, thromboxane.

(3 mg P.O. [oral administration]) inhibits *ex vivo* ADP- and collagen-induced platelet aggregation in dogs.[3]

Cilostazol inhibits not only platelet aggregation but also other aspects of platelet activation, such as thromboxane B_2 (TXB_2) production, PDGF release, expression and release of P-selectin (CD62P), platelet–leukocyte interaction, and microparticle generation.[20,25–28] Effects of cilostazol on platelets are reversible, depending on the blood concentration of cilostazol. In contrast, the effects of aspirin (see Chapter 60) and clopidogrel (see Chapter 61) on platelets are irreversible. Cilostazol might attenuate the responsiveness of platelets to various agonists.

The antiaggregation effect of cilostazol is enhanced in the presence of PGE_1 and adenosine, which activate adenylate cyclase.[5,29] This antiplatelet effect is also enhanced in the presence of cultured vascular endothelial cells.[25] This latter phenomenon is the result of a synergistic effect between the activation of cAMP synthesis by PGI_2 generated from vascular endothelial cells and the inhibition of cAMP hydrolysis by cilostazol.

These antiplatelet effects of cilostazol have been demonstrated in patients with thrombotic diseases. In patients with cerebral infarction or arteriosclerosis obliterans, cilostazol (100–200 mg/day) inhibited ADP-, collagen-, arachidonic acid-, and epinephrine-induced *ex vivo* platelet aggregation.[30,31] In patients with cerebral arteriosclerosis, cilostazol (100 mg/day for 2 weeks) decreased plasma TXB_2 levels.[32] Unlike aspirin, cilostazol does not decrease endothelial cell-derived PGI_2 levels. In patients with cerebral thrombosis or cerebral arteriosclerosis, cilostazol (100 mg/day for 4 weeks) significantly decreased plasma β-thromboglobulin and platelet factor 4 levels.[33] In patients with type II diabetes, cilostazol (150 mg/day for 4 weeks) decreased the levels of platelet activation markers such as serum-soluble vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and P-selectin; platelet-derived microparticles; platelet surface markers P-selectin and CD63; and platelet membrane-expressed annexin V.[34] Using flow cytometry analysis of whole blood obtained from the coronary sinus of patients undergoing coronary stenting, the stent-induced increase in platelet surface P-selectin expression and the increase in neutrophil Mac-1 (CD11b) expression were suppressed in the cilostazol group (200 mg/day) compared to the ticlopidine group (200 mg/day).[35]

IV. Antithrombotic Effects

By virtue of the antiplatelet effects described previously, cilostazol has been shown to have antithrombotic effects *in vivo*. Cilostazol inhibited ADP- and collagen-induced pulmonary thrombotic embolism and reduced mortality in mice at doses of 10 and 3 mg/kg P.O., respectively, with more potent effects than aspirin and pentoxyfylline.[3] In the laser-induced thrombosis model with mouse cremaster artery (see Chapter 34), cilostazol (10 mg/kg intravenously [IV]) inhibited platelet accumulation at the site of vascular injury site.[36,37] Analysis of the kinetics of individual platelets at injury sites using intravital microscopy demonstrates that cAMP directs the rate at which platelets attach to and detach from thrombi. This study demonstrated that cAMP in circulating platelets controls attachment to and detachment from sites of arteriolar injury.

In rabbits with cerebral infarction induced by injection of arachidonic acid into the unilateral internal carotid artery, cilostazol (1 mg/kg IV) reduced the area of infarction identified by perfusion with India ink by 55%.[38] In canine models of peripheral circulatory insufficiency in a hind leg caused by injection of lauric acid into the peripheral end of the ligated femoral artery, cilostazol (10 mg/kg/day P.O.) inhibited the progression of ischemic ulcers and the decrease in skin temperature in obstructed hindlimbs.[39] In dogs, cilostazol (100 mg/kg/day P.O.) inhibited thrombotic occlusion in an artificial vessel transplanted as a replacement for the femoral artery.[40] The efficacy of cilostazol in preventing abrupt reocclusion after percutaneous coronary intervention (PCI) was examined in dogs.[41,42] In this model, Saitoh et al. examined the efficacies of antiplatelet drugs in inhibiting abrupt platelet thrombus reformation after tissue plasminogen activator dissolved thrombin-induced thrombi in a coronary artery, in which high shear stress is also important. Reocclusion occurred in six of the seven animals given aspirin (35 mg/kg IV), a result not significantly different from that of the control group. Similarly, beraprost (12 μg/kg IV) failed to prevent reocclusion in five of seven animals. However, cilostazol (1.8 mg/kg IV) prevented reocclusion in six of seven animals. These antithrombotic effects of cilostazol clearly reflect its antiplatelet properties. It is suggested that cilostazol might have synergistic effect with PGI_2 generated from endothelial cells, as described previously. It is therefore possible that cilostazol may have a more potent inhibitory effect on platelet function *in vivo* than *in vitro* and *ex vivo*.

V. Other Effects

A. *Vasodilation*

In vascular smooth muscle cells, PDE1, 3, 4, and 5 are found at the protein level and PDE1A, 1B, 1C, 3A, 4B, 4D, and 5A are found at the mRNA level.[43–45] The vasodilating effect of cilostazol, similar to its effect on platelet aggregation, is due to an increase in intracellular cAMP levels caused by inhibition of PDE-3A activity in vascular smooth muscle cells.[46] Cilostazol induces the relaxation of rabbit mesenteric artery strips precontracted by KCl. Cilostazol dilates precontracted guinea pig cerebral basilar arteries.[47] The response to cilostazol is independent of the endothelium and of the NO–cGMP pathway in cerebral arteries. In anesthetized dogs,

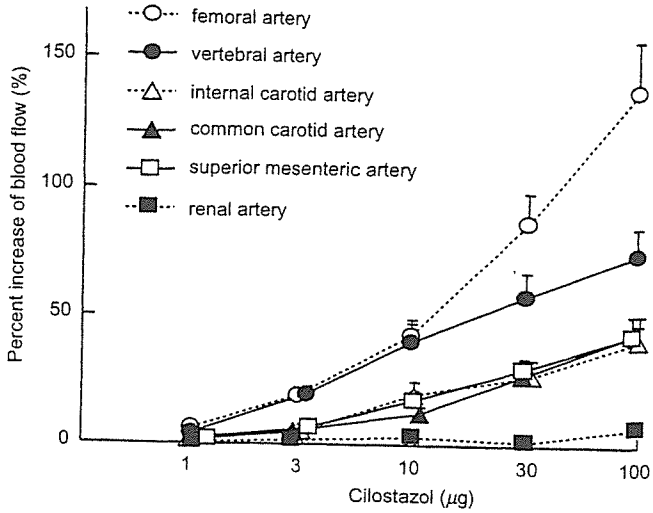

Figure 64-3. Vasodilating effects of cilostazol by intraarterial administration in anesthetized dogs. Data are mean ± SE ($n = 5$).

intraarterially administered cilostazol dose dependently increased the blood flow in the vertebral artery, the internal carotid artery, and the common carotid artery (Fig. 64-3).[48] This vasodilatory effect of cilostazol was particularly pronounced in the vertebral artery and the femoral artery, but it was minimal in the renal artery. Intravenously administered cilostazol also significantly increased cerebral and femoral arterial blood flow in dogs.

In patients with chronic arterial occlusion in the lower extremities, blood flow to the lower extremities significantly increased after administration of cilostazol 150 to 200 mg/day for 2 weeks,[49,50] and skin blood flow was also increased by cilostazol 200 mg/day for 6 weeks.[51] In a double-blind, randomized, crossover study, cilostazol (200 mg) or placebo was administered orally to 12 healthy participants.[52] Mean flow velocity in the middle cerebral arteries (MCA) was measured with transcranial Doppler (TCD), and the diameters of the superficial temporal and radial arteries were measured by ultrasonography. Velocity in the MCA decreased 21.5 ± 5.7% after cilostazol and 5.5 ± 12.2% after placebo ($p = 0.02$ vs. placebo), without any change in global or regional cerebral blood flow. The superficial temporal artery diameter increased 17.6 ± 12.3% ($p < 0.001$ vs. baseline) and radial artery diameter increased 12.6 ± 8.6% ($p < 0.001$ vs. baseline). This study suggested that cilostazol dilates the MCA without affecting cerebral blood flow or blood pressure.

B. Inhibition of Vascular Smooth Muscle Cell Proliferation

In a study that assessed [^{3}H] thymidine uptake and cell counting, cilostazol inhibited proliferation of rat aortic smooth muscle cells in culture.[53] Cilostazol potently inhibited [3H] thymidine uptake by smooth muscle cells stimulated by various growth factors, such as PDGF and insulin, and exhibited no growth factor specificity. PDE3 inhibitors, including cilostazol, also inhibit smooth muscle cell proliferation and were found to inhibit intimal hyperplasia in rat and mouse models of vascular injury.[54–57] Their inhibitory effects on intimal hyperplasia are considered dependent on their inhibition of cell proliferation by increasing cAMP levels in vascular smooth muscle cells. Clinically, cilostazol also significantly decreased the incidence of intimal hyperplasia and restenosis after PCI, directional coronary atherectomy, and stent implantation, as described in Section VI.

C. Cytoprotective Effects

Cilostazol has cytoprotective effects on cultured endothelial cell (EC) dysfunction. Through mediation of the stimulation of hepatocyte growth factor production, cilostazol prevents EC death induced by high glucose or hypoxia.[56,58] Cilostazol significantly attenuates the dose-dependent increment of monocyte chemoattractant protein-1 production by tumor necrosis factor (TNF)-α.[59] Cilostazol also prevents EC death induced by lipopolysaccharide (LPS).[60] Cilostazol reduces the increases in TNF-α production, Bax protein expression, and cytochrome c release induced by LPS, and it reverses the decrease of Bcl-2 protein. Cilostazol suppresses remnant lipoprotein particle-induced apoptosis of EC.[61]

To investigate the effects of cilostazol on hemispheric ischemic lesions, the apparent diffusion coefficient (ADC) and T2 images by magnetic resonance imaging (MRI) techniques were compared with histology at the termination of and 24 hours after reperfusion following a 2-hour occlusion of rat MCA.[62] Cilostazol (30 mg/kg P.O. at 5 minutes and 4 hours after reperfusion) significantly suppressed the hemispheric lesion area and volumes when detected by ADC, T2 images, and histology. Cilostazol significantly reduced the increased cerebral water content at the ischemic hemisphere. The neurological deteriorations were much improved in the cilostazol-treated group. These investigators[62] suggested that posttreatment with cilostazol exerts a potent protective effect against cerebral infarct size by reducing the cytotoxic edema.

D. Effect on Lipid Metabolism

By improving lipid metabolism, cilostazol decreases plasma triglycerides and remnant lipoprotein cholesterol, and it increases high-density lipoprotein cholesterol levels in patients with peripheral arterial diseases and those with type 2 diabetes mellitus.[63–66] Cilostazol increases apolipoprotein A_1 and decreases apolipoprotein B levels without affecting the low-density lipoprotein cholesterol level.

The lipid metabolism-improving effect of cilostazol is considered to be due to enhanced lipoprotein lipase activity[67] and is particularly pronounced in patients with hypertriglyceridemia.

VI. Clinical Results

By virtue of the pharmacological effects described previously, cilostazol has been shown to be effective in various clinical disorders of thrombosis and circulatory insufficiency.

A. Prevention of Recurrence of Cerebral Infarction

To evaluate the efficacy of cilostazol in preventing recurrent cerebral infarction, a multicenter, double-blind, placebo-controlled trial — the Cilostazol Stroke Prevention Study (CSPS) — was performed from 1992 to 1996 and included 1095 patients.[68,69] Patients were randomized to treatment with cilostazol (100 mg twice daily) or placebo for at least 1 year and up to 5 years (mean treatment period, 632 days). The primary outcome of recurrence of cerebral infarction (fatal and nonfatal) is shown in Fig. 64-4. Cerebral infarction recurred in 30 of 526 patients (5.7%) receiving cilostazol and in 57 of 526 patients (10.8%) receiving placebo. This difference was statistically significant ($p = 0.0149$), with a relative risk reduction of 41.7%. The annual recurrence rate was 3.37% in the cilostazol group and 5.78% in the placebo group.

In the CSPS trial, there were four major hemorrhagic events (three cerebral hemorrhages and one subarachnoid hemorrhage) in the cilostazol group and seven (cerebral hemorrhages) in the control group. Adverse events of bleeding tendency excluding the previously mentioned major hemorrhagic events were reported in 2.8% (15/526) of the cilostazol-treated patients and 2.1% (11/526) of the controls, a nonsignificant difference. These outcomes concerning bleeding events are noteworthy because clinical studies on other antiplatelet drugs have shown a significant difference in the rate of hemorrhagic events between the placebo and the antiplatelet drug-treated groups. In a small study,[70,71] cilostazol (200 mg/day), aspirin (330 mg/day), and ticlopidine (300 mg/day) were administered to 10 healthy men for 3 days at the respective doses found to have an antiplatelet effect. Pre- and postdrug administration, bleeding time, and volumes were measured with a quantitative bleeding time test apparatus. A small incision (1 mm deep and 1 cm long) was made on the forearm by the Simplate and was covered by a small cup. Physiological saline was continuously perfused into the cup. The amount of hemoglobin from the incision was continually and quantitatively measured to calculate bleeding time and volume. Bleeding time changed as follows (predrug to postdrug): 359.0 ± 95.8 to 646.0 ± 248.0 seconds for aspirin, 323.3 ± 99.9 to 528.7 ± 180.2 seconds for ticlopidine, and 313.0 ± 112.5 to 343.3 ± 154.0 seconds for cilostazol. Bleeding volumes before and after administration of aspirin, ticlopidine, and cilostazol were 14.5 ± 4.9 and 30.2 ± 18.8 μL, 12.5 ± 5.0 and 19.2 ± 7.2 μL, and 12.4 ± 5.2 and 13.4 ± 6.8 μL, respectively. Both bleeding time and volume were significantly changed after administration of aspirin and ticlopidine. By contrast, cilostazol at a dose found to show the same antiplatelet effect as aspirin and ticlopidine in the same study did not significantly change bleeding time or volume.

The effect of cilostazol on the progression of intracranial arterial stenosis (IAS) was investigated in 135 patients with acute symptomatic stenosis in the M1 segment of MCA or the basilar artery randomized to either cilostazol (200 mg/day) or placebo for 6 months.[72] Aspirin (100 mg/day) was given to all patients. IAS was assessed by magnetic resonance angiogram and TCD at the time of recruitment and 6 months later. In the cilostazol group, 3 (6.7%) of 45 symptomatic IASs progressed and 11 (24.4%) regressed. In the placebo group, 15 (28.8%) of symptomatic IASs progressed and 8 (15.4%) regressed. Progression of symptomatic IASs in the cilostazol group was significantly lower than that in the placebo group ($p = 0.008$).

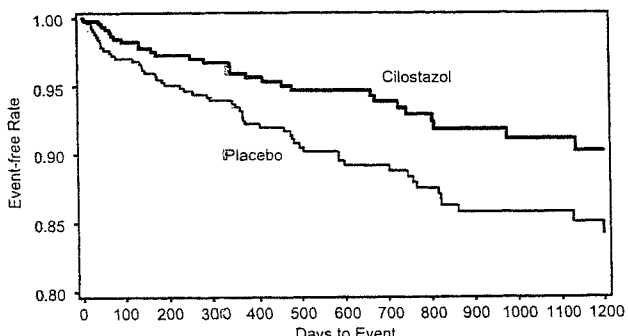

Figure 64-4. Kaplan–Meier plot for the primary outcome (recurrence of cerebral infarction) according to assigned treatment in the Cilostazol Stroke Prevention Study (CSPS). The numbers of patients at risk are shown at the bottom.

B. Intermittent Claudication in Chronic Arterial Occlusion

Using the primary end point of maximum walking distance on a treadmill, the efficacy of cilostazol for the treatment of intermittent claudication due to lower limb blood flow insufficiency has been evaluated in the United States since 1993.[73,74] In a clinical trial for this purpose, the absolute claudication distance (ACD) was measured in 239 patients

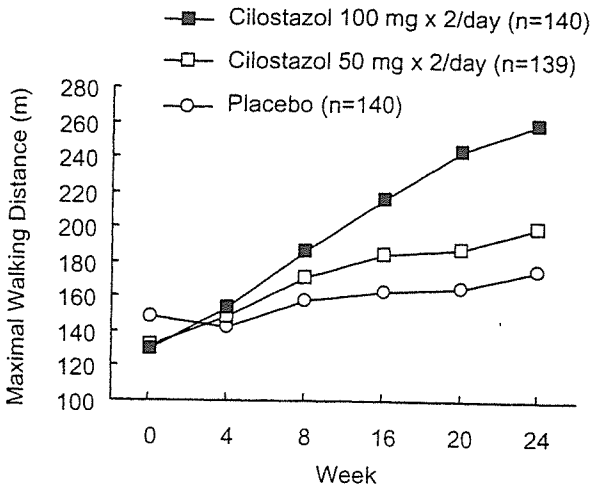

Figure 64-5. Effect of cilostazol on maximal walking distance in patients with intermittent claudication. Data are from Beebe et al.[75]

with chronic peripheral arterial occlusion who received cilostazol (200 mg/day) or placebo for 12 weeks.[74] ACD increased by 47.0% in the cilostazol group (119 patients) and by 12.9% in the placebo group (120 patients) ($p < 0.001$). This result appears to be associated with cilostazol's vasodilatory effect on the femoral artery. Based on these results and those of seven other large-scale clinical trials (Fig. 64-5),[73–76] oral cilostazol was granted approval by the FDA in January 1999 for the treatment of various symptoms in patients with intermittent claudication.

A meta-analysis of the results from these eight randomized, placebo-controlled trials has been performed.[77] The meta-analysis examined the effect of cilostazol on pain-free and maximal walking distance, quality-of-life (QOL) measures, and adverse effects in 2702 patients with stable, moderate to severe claudication. Treatment ranged from 12 to 24 weeks. Cilostazol therapy increased maximal and pain-free walking distances by 50 and 67%, respectively. In subgroup analysis, cilostazol increased pain-free and maximal distances similarly in men and women, in older and younger patients, and in patients with and without diabetes. QOL assessments revealed enhanced scores for physical well-being. Cilostazol-treated patients reported a higher incidence of headache, bowel complaints, and palpitations than patients given placebos. This meta-analysis demonstrated that cilostazol significantly increases walking distances and QOL measures in patients with claudication without major adverse effects.

C. Restenosis after PCI and Stent Implantation

Restenosis after PCI and stenting appears to be the result, at least in part, of proliferation of vascular tissues.[78] In most clinical trials, aspirin and ticlopidine have not shown a clear benefit in preventing this type of restenosis. However, cilostazol has been demonstrated in many studies to prevent restenosis through its inhibitory effect on vascular smooth muscle cell proliferation. Kunishima et al.[79] studied the effect of cilostazol and aspirin on restenosis approximately 5 months after stent implantation. The minimal lumen diameter (MLD) at follow-up was 2.34 ± 0.74 mm in the cilostazol group (200 mg/day, $n = 28$ patients with 35 lesions) and 1.89 ± 1.08 mm in the aspirin group (81 mg/day, $n = 37$ patients with 41 lesions), revealing significant dilation in the cilostazol group. The restenosis rate was 8.6% in the cilostazol group compared to 26.8% in the aspirin group. This study suggests that administration of cilostazol alone after the implantation of an intracoronary Palmaz–Schatz stent is useful for the prevention of restenosis.

Tsuchikane et al.[80] studied the effect of cilostazol and aspirin on restenosis 3 months after PCI. Late loss, which was calculated as the difference between post-PCI MLD and MLD at follow-up angiography, was 0.15 ± 0.45 mm in 123 patients treated with cilostazol (200 mg/day) and 0.45 ± 0.52 mm in 129 patients treated with aspirin (250 mg/day) ($p < 0.0001$). The angiographic restenosis rate, defined as follow-up diameter stenosis exceeding 50%, was significantly lower in the cilostazol group (17.9 vs. 39.5%, $p < 0.0001$), which demonstrated efficacy of cilostazol in preventing restenosis.

In a study conducted by Ochiai et al.,[81] aspirin (81 mg) was administered to all patients who received primary Palmaz–Schatz stenting within 12 hours after acute myocardial infarction, and the patients were randomized to receive cilostazol (200 mg/day for 6 months) or ticlopidine (200 mg/day for 1 month) to prevent subacute stent thrombosis. Clinical and angiographic outcomes at 6 months were analyzed. Late loss was 0.49 ± 0.40 mm in the cilostazol group and 0.88 ± 0.52 mm in the ticlopidine group. Restenosis rates were 0 and 20%, respectively ($p = 0.05$), demonstrating the inhibitory effect of cilostazol on restenosis.

In another study of patients who were implanted with a Palmaz–Schatz stent, 56 patients received cilostazol 200 mg/day and 58 patients received ticlopidine 200 mg/day (the standard dose in Japan) for 6 months.[82] Late loss in the cilostazol and ticlopidine groups was 0.58 ± 0.52 and 1.09 ± 0.65 mm, respectively ($p < 0.0001$). The restenosis rate was significantly lower in the cilostazol group (16 vs. 33%, $p = 0.0044$). Analysis of outcome at 6 months (total 1-year follow-up) showed that the target vessel revascularization rate at 1 year was 23% in the cilostazol group and 42% in the ticlopidine group ($p = 0.03$), also suggesting that cilostazol is effective in the prevention of restenosis after stenting. Other studies have also reported this inhibitory effect of cilostazol on restenosis after coronary intervention.[83–85]

The Cilostazol for Restenosis (CREST) trial is in progress to evaluate more definitively the ability of cilostazol to

prevent restenosis following uncomplicated stent implantation for *de novo* coronary artery stenosis.[86,87] In this randomized, double-blind, multicenter study, 700 patients will receive clopidogrel, aspirin, and either cilostazol or placebo after successful intracoronary stent implantation. The primary end point is MLD of the first lesion stented after 6 months; secondary end points include MLD in all lesions, mean percentage diameter stenosis, target lesion revascularization, and major angiographic end points.

D. Adverse Effects

Side effects are infrequent with cilostazol, but they include headache, palpitations, and diarrhea. Cilostazol is contraindicated in patients with congestive heart failure. The lack of cilostazol-induced hemorrhagic side effect in the 1095-patient CSPS study is discussed in Section VI.A.

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