PLATELETS

Review

The Basic Pharmacology of Ticlopidine and Clopidogrel

K. Schrör

SUMMARY. **Ticlopidine and clopidogrel are two thienopyridines with potent and apparently irreversible platelet inhibitory properties. The antiplatelet effects are mainly directed against ADP-induced stimulation of platelet function, in particular ADP-induced inhibition of adenylyl cyclase stimulation. There is evidence for additional effects, including inhibition of agonist-induced intracellular Ca^{++} mobilization, interference with GpIIb/IIIa receptor/agonist interaction and inhibition of α-granule secretion. However, these actions are probably secondary to the ADP-antagonistic action.**

Thienopyridines do not directly interfere with arachidonic acid metabolism. The substances are inactive in vitro and have to undergo some form of bioactivation in vivo which requires 3 to 5 days of treatment for a maximum effect. The nature of the postulated active metabolite(s) is still unknown. From a pharmacological point of view, thienopyridines may be considered interesting alternatives to acetylsalicylic acid with particular value in shear-stress-mediated platelet activation, for example in prevention of acute thrombembolic risk in injury-related vessel stenosis.

More than 15 years ago, the first report on the antiplatelet actions of a thienopyridine, ticlopidine, in man was published.[1] The report contained important information on this compound: selective inhibition of ADP-induced platelet aggregation, including (1) inhibition of ADP-induced primary aggregation, i.e. platelet activation that is independent of thromboxane pathways and secretion of storage products; (2) a maximum effect only after 5 to 6 days of repeated oral treatment, and (3) persistence of this effect for several days after drug withdrawal, suggesting that this action was essentially irreversible. Although these findings resembled those seen after treatment with acetylsalicylic acid (ASA), it was obvious that marked differences existed, in particular the inhibition of ADP-induced primary aggregation which is not seen with ASA and related compounds.

K. Schrör, MD, Institut für Pharmakologie, Heinrich-Heine-Universität Düsseldorf, Moorenstr. 5, D-40225 Düsseldorf, Germany. Tel: (211) 311-2500; Fax: (211) 311-4781.

Since then, a multitude of experimental and clinical trials have been published that confirm a potent, ADP-selective and apparently irreversible antiplatelet action of ticlopidine and related thienopyridines such as clopidogrel (see refs 2–4 for reviews). Ticlopidine has also been studied in several clinical trials, in particular in patients with ischemic cerebrovascular diseases, and has been found to possess considerable clinical efficacy (for review see refs 3–4).

The basic pharmacology of the compound is not yet completely understood. In particular, the precise mechanism of action of thienopyridines is still unknown. This is at least partially due to an incomplete understanding of the platelet ADP receptor and its signal transduction coupling.[6] Ticlopidine is inactive in vitro at concentrations below 0.1 mM[5] and has to undergo a bioactivation to an unknown metabolite in vivo to exert its full antiplatelet potential.

This review summarizes the basic pharmacology of the two clinically used thienopyridines on platelet

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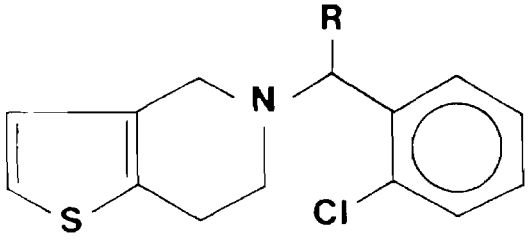
function, i.e. ticlopidine and clopidogrel. Consideration is given to the fresh insights that have come from a better understanding of the mechanisms of ADP-induced platelet activation.

Chemistry

Ticlopidine {5-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2:c]-pyridine} (Fig. 1), available as the hydrochloride, is a thienopyridine without apparent structural relation to other known antiplatelet agents. Substitution at the benzyl carbon leads to PCR 4099 {methyl 2-(2-chlorophenyl)-2-(4,5,6,7-tetrahydrothieno-[3,2:c]-pyridin-5-yl)-acetate}. In contrast to ticlopidine, PCR4099 is a racemate, of which the stereoisomeric forms (*S* - SR25990c - clopidogrel: *R* - SR25989c) have been separated and are generally available as the hydrogen sulphates. All the desired biological activity resides in the dextrorotatory (*S*) form.[7] Clopidogrel differs from ticlopidine in its higher molar potency and equipotent oral and intravenous activity.[92] With respect to antiplatelet actions ticlopidine and clopidogrel, as well as the racemate, PCR 4099, appear to behave identically and are considered together.

Actions of Thienopyridines on the Hemostatic System

Within the hemostatic system, thienopyridines appear to selectively modify platelet function.[2–5] Ticlopidine is inactive in conventional coagulation and fibrinolysis assays. There is a minor reduction (by 9–13%) of blood viscosity in rats[8] and also one report, showing that long-term treatment with ticlopidine may reduce hematocrit and plasma fibrinogen levels.[9] However, most investigators agree that the prolongation of bleeding time by thienopyridines in vivo is mainly if not entirely due to inhibition of platelet function.



R = H : TICLOPIDINE

R = $COOCH_3$: PCR 4099

Fig. 1 Structures of ticlopidine and PCR 4099.

Selectivity of Thienopyridines for ADP

ADP has been known for more than 30 years to be a potent stimulus of platelet function.[10] ADP also belongs to the group of most abundant and significant endogenous mediators that promote platelet activation at a site of vessel injury in vivo.[11] Ticlopidine and clopidogrel appear to be specific antagonists of both primary and secondary phases of platelet aggregation induced by ADP. Ticlopidine does not inhibit platelet cyclooxygenase or thromboxane synthase at antiplatelet doses and also has no effect on vascular prostacyclin production.[12–13] These properties, as well as the inhibition of ADP-induced primary aggregation by thienopyridines, are different from the effects of ASA and related compounds. There is some effect of ticlopidine on the aggregation induced by other platelet agonists, including arachidonic acid, thromboxane mimetics, collagen, thrombin, PAF and stimulators of protein kinase C. However, these inhibitory effects of ticlopidine in most studies are abolished by increasing the agonist concentration and thus are probably due to blocking the contribution of ADP released from endogenous sources to the platelet stimulation by these agonists.[4–5 14–15]

The inhibitory potential of thienopyridines is similar for exogenously added ADP as well as for ADP released from endogenous sources during platelet secretion.[14] Ticlopidine treatment does not inhibit potentiation of ADP-induced platelet aggregation by adrenaline.[15–17] This is interesting because it suggests that any interaction of ticlopidine with the ADP receptor will not influence receptor sensitization by catecholamines.

The interaction of ADP with platelets produces various effects including specific receptor binding, adenylyl cyclase inhibition, increased cytosolic Ca^{++} and ADP-induced exposure of the platelet fibrinogen receptor as a final common pathway of all types of platelet agonists.[18] Thus, the question arises which of these mechanisms is selectively affected by ticlopidine.

ADP-receptors

Platelets contain considerable amounts of ADP in their storage granules (see ref. 19 for review) which is released during platelet secretion and probably serves as an amplification mechanism for platelet aggregation. ADP cannot penetrate the intact cell membrane. Thus, the actions of ADP on platelet function require specific binding sites at the cell surface membrane.

If thienopyridines directly interfere with ADP receptors, then they should interfere with ADP binding to its high-affinity binding site(s) at the platelet membrane. Although early studies, using radiolabelled ADP as ligand, have identified an ADP binding protein in solubilized platelet membranes,[20] it is not clear whether this binding protein functions

as an ADP receptor and mediates the action of ADP on platelet function. It should be noted that ADP is rapidly metabolised, taken up and released by platelets.[21] Metabolic degradation of ADP may result in formation of adenosine which can antagonize the inhibitory effect of ADP on adenylyl cyclase.[21] All this makes it difficult to maintain stable equilibrium concentrations of ADP in the extracellular space which, however, is a prerequisite for ligand-binding studies.

More detailed information about the nature of platelet ADP receptor(s) and their signal transduction pathways was obtained after more selective and stable ADP-receptor ligands became available.[22–27] Studies with the structural analogue (5'-p-fluorosulfonylbenzoyladenosine)[23] showed that ADP mediated platelet shape change, aggregation and exposure of the fibrinogen receptor could be selectively blocked by this agent while the effects of ADP on cAMP formation remained unchanged. The effects of ADP on adenylyl cyclase and on storage-dependent Ca^{++} release from platelets were selectively blocked by another structural analogue, 2-methylthio-ADP, (2-MeSADP). This effect was also inhibited by ADP.[24] These data and related findings suggested that two different binding sites are involved that mediate the different actions of ADP. However, the close linear relationship between modification of platelet function and changes in cyclic AMP formation which was found for a series of 7 structurally related ADP analogues[25] suggested a single common class of ADP receptors. More recently, a high- and low-affinity binding site for ADP could be separated in paraformaldehyde fixed platelets. These platelets do not metabolize ADP. The high affinity binding site was occupied by a number of agonists and antagonists but not by adenosine.[26] Recent work by the same group,[27] using photoaffinity-labelled nucleotides, showed that the platelet ADP receptor has low structural specificity and is also occupied by other nucleotides, including GDP and GDPβS. This is an interesting finding, because it suggests that inhibition of platelet function by GDP may not necessarily indicate a role for G-proteins in signal transduction.[27]

Ticlopidine does not modify the number or activity (k_D) of high- and low-affinity binding sites for ADP in formaldehyde-fixated human platelets.[14] As already mentioned, the possibility should not be excluded that formaldehyde fixation may abolish any effect of ticlopidine on the ADP receptor. No change in ADP high-affinity binding sites but reduced low-affinity binding was found using ADP as ligand.[28] However, in this study the ADP may have been metabolized by intact platelets. In another study on platelets prepared from patients with vascular diseases, pretreatment with clopidogrel reduced the number of binding sites for 2-MeSADP by 63%. This was associated with a 3-fold increase in the EC_{50} for inhibition of ADP-induced inhibition of cAMP accumulation.[29] From these data is was concluded that clopidogrel selectively reduces the number of functional receptors that mediate inhibition of stimulated adenylyl cyclase activity by ADP.[30] Studies in rat platelets have shown that clopidogrel impairs the interaction of the ADP receptor with its, yet unidentified, G-protein, eventually by an irreversible modification of the putative G-protein. This effect of clopidogrel was again restricted to stimulation by 2-MeSADP, i.e. the ADP receptor ligand that binds to the subtype mediating decrease in platelet cAMP.[24,32]

Taken together, most of these data are compatible with the hypothesis that clopidogrel and ticlopidine selectively inhibit expression, occupation or function of the MeSADP-binding ADP receptor subtype(s) on the platelet membrane that mediate the inhibition of adenylyl cyclase activity by ADP.[29] This is schematically shown in Figure 2.

Cyclic AMP

ADP inhibits stimulated human platelet adenylyl cyclase while the ADP receptor antagonist ATP causes a non-competitive inhibition of the enzyme.[24,33–34] The k_i is 0.3 μM and complete inhibition is obtained at 2 μM. These concentrations of ATP are well within the physiological range (see below).

Ticlopidine in vitro does not inhibit phosphodiesterase in platelets,[5] does not interfere with adenosine uptake, and does not directly stimulate platelet adenylyl cyclase in intact platelets. In most[16,36–39] but not all studies,[35] basal cAMP levels were unaffected or only modestly elevated by ticlopidine. However, all available studies agree that thienopyridines ex vivo antagonize ADP-induced inhibition of stimulated adenylyl cyclase activity and that this results in significantly elevated cAMP levels,[16,35–39] pointing to modulation of adenylyl cyclase as an important determinant of the antiplatelet actions of thienopyridines. This effect is stereospecific[39] for clopidogrel and not seen with its enantiomer SR 25989C. This action of thienopyridines is most profound for ADP-induced reduction in cAMP generation, and not seen after platelet stimulation with adrenaline.[36,39] This agrees with the previous notion that ticlopidine does not affect the adrenaline-induced sensitization of platelet to ADP.[15–17] There is one report[5] showing that treatment of human platelets with an inhibitor of adenylyl cyclase (SQ 22536) did not antagonize the inhibition of platelet function by ticlopidine. It was concluded that ticlopidine does not require elevated cAMP for its platelet inhibitory action. However, no positive evidence that the adenylyl cyclase inhibitor was active in this study was presented and cyclic AMP levels were not measured.

In the rat, clopidogrel not only antagonized the reduction in platelet cAMP induced by ADP but also

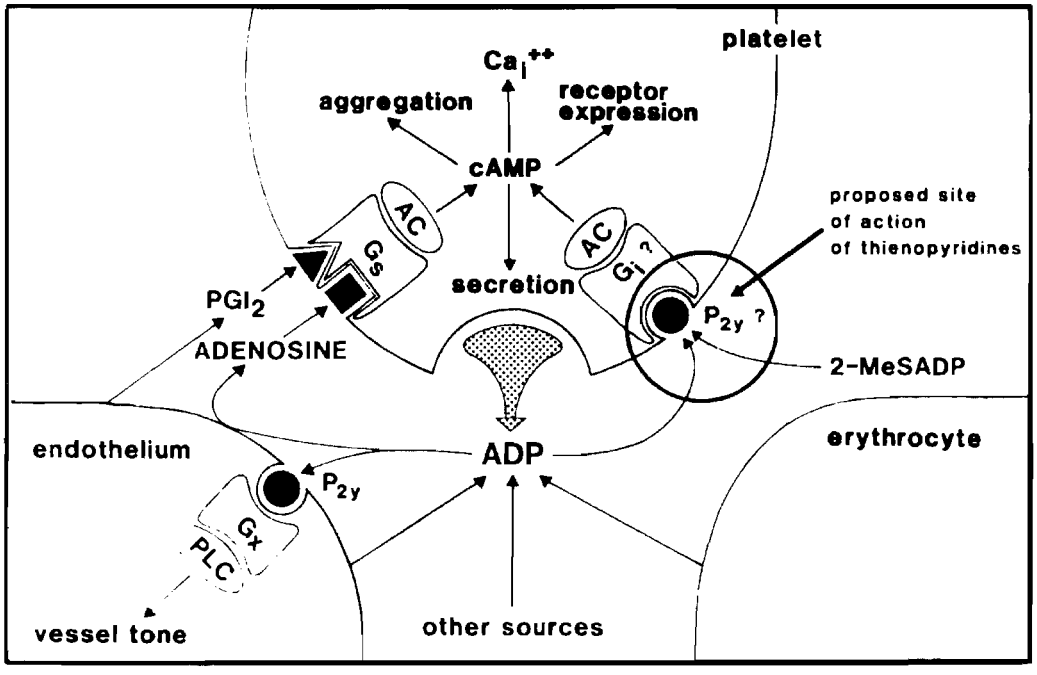


Fig. 2 Proposed mechanism and site of action of ticlopidine. Ticlopidine interferes with signal transduction via the ADP-receptor subtype (P_{2y}?) that inhibits stimulation of platelet adenylyl cyclase. This results in an enhanced cAMP level, in particular in the presence of adenylyl cyclase stimulating compounds, such as adenosine or PGI_2. Further explanation see text. PLC: phospholipase C, G: G-protein; AC: adenylyl cyclase; Ca_i^{++}: cytosolic Ca^{++}.

that following stimulation by thrombin. This effect of thrombin was prevented by apyrase, suggesting that endogenous ADP was involved.[39] Interestingly, there was no modification of thrombin-induced inhibition of cAMP accumulation in fawn-hooded rats. These animals have a congenital deficiency of ADP in their dense granules. This is another argument that the release of ADP from endogenous sources is necessary to mediate this action of clopidogrel. However, cyclic AMP-mediated responses to PGE_1 can also be partially antagonized by a thromboxane mimetic (U 46619)[40] and, eventually, other platelet stimulatory compounds. This could be due to an inhibition of adenylyl cyclase. However, release of endogenous ADP may also mediate these actions. In other words, inhibition of platelet agonist-induced reductions in cAMP will be less pronounced if endogenous ADP is not a major contributing factor to platelet stimulation.

Direct evidence for this hypothesis was provided in a recent study of ADP-induced platelet aggregation and cAMP accumulation in cholesterol-fed rabbits.[41] As expected, ticlopidine treatment resulted in a significant stimulation of hormonal (iloprost)-stimulated platelet cAMP accumulation and inhibition of ADP-induced platelet aggregation. Inhibition of platelet aggregation by ticlopidine was markedly attenuated during cholesterol feeding (Fig. 3). In cholesterol-fed animals, there was a biphasic ADP-induced aggregation and significant ADP-induced thromboxane formation.[42] Both events did not occur in normal rabbits. These data suggest that ticlopidine may be a less effective though still active inhibitor of ADP-induced platelet activation in hypercholesterolemia, i.e. in conditions when enhanced ADP-induced thromboxane formation occurs and largely mediates the platelet stimulatory action of ADP.

Cytosolic Ca^{++}

One of the mechanisms by which cAMP regulates platelet function is by controlling the level of Ca^{++} in the cytosol.[43] Any significant rise in cAMP will antagonize the ADP-mediated release of Ca^{++} from intracellular stores but not the influx from the extracellular space.[44–45] In this context it is important to note that ADP differs from other platelet stimulatory agonists, e.g. thrombin, because it directly stimulates Ca^{++} entry from the extracellular space before any release from internal stores becomes detectable.[46] The early rise in Ca^{++} in aspirin-treated platelets does not require the generation of second messengers such as IP_3 or diacylglycerol from phosphatidylinositol (PI)-dependent pathways.[44–47] Similar data were obtained by Vickers et al[48] who additionally demonstrated that ADP may stimulate PI-metabolism in non-ASA-treated platelets, in particular when secondary aggregation occurred at low external Ca^{++} concentrations. These findings, as well as data from Sweatt et al,[49] suggest that the secondary stimulation of the PI-response by ADP is

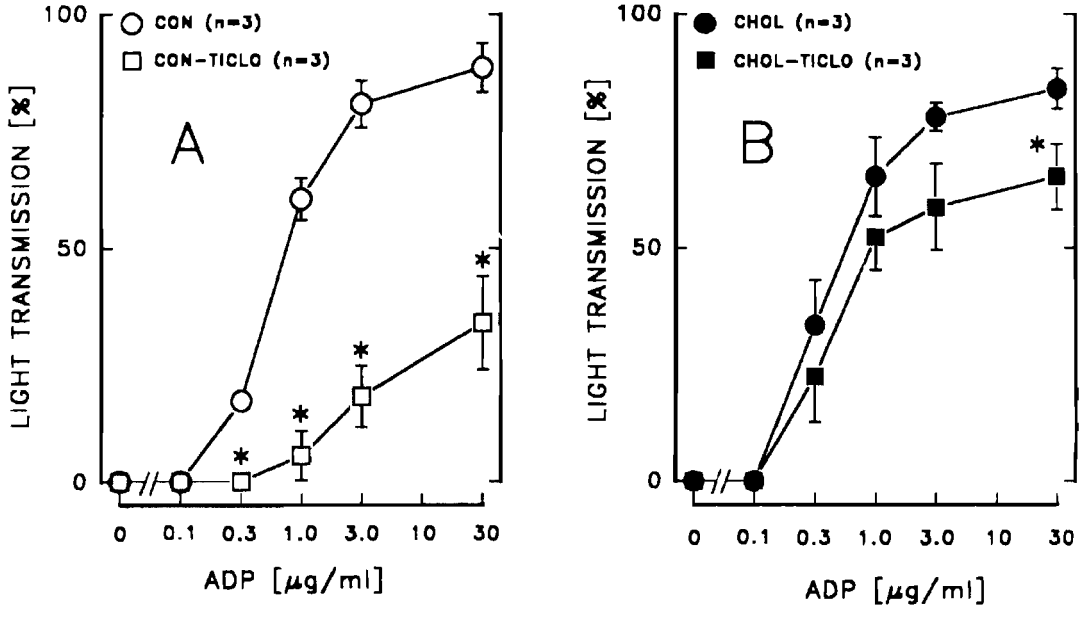


Fig. 3 Actions of oral ticlopidine (TICLO) on ADP-induced platelet aggregation in rabbits fed a cholesterol-rich diet for 4 months. Animals were untreated (CON) or treated with ticlopidine (100 mg/kg × day). (*): $P < 0.05$ (treatment vs control).

thromboxane/PG-endoperoxide-mediated. This agrees well with the original observation by Charo et al[50] that there is a requirement for prior aggregation, i.e. indomethacin-sensitive thromboxane formation, for ADP-induced platelet secretion.

A more detailed insight into the cellular signal transduction mechanisms of ADP in platelets came from electrophysiological patch-clamp experiments.[51–52] It was shown that the primary, ADP-induced stimulation of Ca^{++} influx was complete within 30 to 40 msec and not seen in the absence of external Ca^{++} and, therefore, entirely due to Ca^{++} influx from the extracellular space through an ADP-activated divalent cation channel. The second phase was delayed but still detectable in the absence of external Ca^{++} and blocked by agents that stimulate cAMP, such as forskolin. Elevation of cAMP did not inhibit the first fast component. These data suggested that store-regulated Ca^{++} entry from internal sources occurs in platelets and that this may be triggered by the ADP-induced initial rise in Ca_i^{++} from the external space.

Ticlopidine did not cause any changes of ADP-induced stimulation of cytosolic Ca^{++} levels in healthy volunteers ex vivo at both physiological external Ca^{++} concentrations (1 mM) and in the absence of external Ca^{++}.[16] Also ticlopidine did not antagonize ADP-induced IP_3 formation in intact platelets nor did it affect IP_3-induced Ca^{++} release from permeabilized platelets.[52–53] However, in another study ticlopidine did antagonize ADP- and thrombin-induced elevation of cytosolic Ca^{++} in the presence of EGTA, i.e. in the absence of external Ca^{++}.[54] Interestingly, ticlopidine and PCR 4099, the racemic clopidogrel, were found to block protein kinase C-induced platelet activation which was assumed to be independent of cytosolic Ca^{++} mobilization.[14] On the other hand, there are studies in rat and rabbit platelets, demonstrating that antiaggregatory thienopyridines inhibit the ADP-induced mobilization of cytosolic Ca^{++} but not the influx of Ca^{++} from the extracellular space.[36,53–54] The inhibition of Ca^{++} mobilization may be due to reduced release from internal stores, subsequent to activation of PI breakdown. This action may be secondary to inhibition of ADP-induced reduction of hormonal-stimulated adenylyl cyclase activity in intact platelets and platelet membranes.

There are several possible explanations for these different findings on thienopyridine-related changes in cytosolic Ca^{++} in animal experiments and man. One is species differences which are very well known for platelet aggregation between rat, rabbit and man. Another is that there are large differences in the doses of ticlopidine used. In animals, usually doses amounting to 100 to 300 mg/kg are applied, but only about 8 mg/kg (250 mg b.i.d.) are given to man. A 20- to 40-fold higher dose in animals, on a weight basis, may have additional effects and perhaps alter tissue distribution of the drug (metabolite(s)). Thus the exact mechanism of changes incytosolic Ca^{++} requires further studies. The message, taken from available data for man, is that inhibition of platelet function by ticlopidine does not require inhibition of ADP-induced rises in cytosolic Ca^{++}, neither from intracellular nor from extracellular sources.

GPIIb/IIIa receptor

In addition to cell surface receptors for chemical agonists (ADP, thrombin, PGE_1/PGI_2 and others) the platelet membrane also contains glycoproteins (GP) of the integrin family. These GP's facilitate the processes whereby normally non-adherent platelets stick to each other and to subendothelial structures in the vessel wall.[18] Aggregation involves the integrin GPIIb/IIIa, the platelet fibrinogen receptor, at the platelet surface. Although formation of small aggregates can also be demonstrated in the absence of fibrinogen,[55] it is the association of fibrinogen with the platelet surface GPIIb/IIIa receptor, probably the α-chain of GPIIb,[27] which is the final common step for platelet aggregation under all natural conditions.[18]

Ticlopidine inhibits ADP-induced exposure of the fibrinogen binding site of the GPIIb/IIIa receptor[15,38,56–57] probably by inducing a defect in the mobilization of the GPIIb/IIIa complex in activated platelets so that its ability to serve as receptor for adhesive macromolecules, such as fibrinogen, is compromised.[18] The binding of fibrinogen to the GPIIb/IIIa receptor induced by several other agonists (thromboxane mimetics, thrombin) is also inhibited by thienopyridines. These effects, like those of ADP, are abolished by ADP scavengers, suggesting that they are all ADP-mediated.[15,38]

Originally it was thought that ticlopidine might interact with the GPIIb/IIIa receptor complex by directly interfering with the fibrinogen binding site.[58] Later studies[15,38,59] have shown that thienopyridines decrease fibrinogen affinity to the 'low affinity' binding site but do not affect the physiologically important high affinity binding site. Specifically, neither ticlopidine nor PCR 4099 affect the GPIIb/IIIa receptor association, its electrophoretic mobility and recognition by different monoclonal antibodies.[38,57,59] Thus, there is substantial evidence for reduced fibrinogen binding after thienopyridine treatment but no evidence of any direct effects of ticlopidine on the platelet fibrinogen receptor complex.[18]

Thienopyridines and Thromboxane Formation

As mentioned above, there is no evidence that thienopyridines directly affect arachidonic acid metabolism. One in vitro report demonstrated arachidonic acid release from platelet membrane phospholipids in the presence of 0.1 to 1 mM concentrations of ticlopidine. This effect was probably due to hydrolysis of phospholipids.[60] Some studies in man have reported that ADP-induced thromboxane release was reduced by ticlopidine,[37,61] whereas others did not detect any alterations.[61,63] In general, changes, if any, were small and probably indirect in nature. A more pronounced effect of ticlopidine was seen, when malondialdehyde (MDA) was measured after thrombin stimulation.[13,35,64–65] However, MDA is not a specific marker for thromboxane formation and inhibition of thrombin binding subsequent to increased cAMP formation[66] may also be considered. Moreover, in contrast to thrombin, there was no change after arachidonic acid stimulation[35] and also no change in serum thromboxane.[64] Therefore, ticlopidine-induced inhibition of ADP-induced platelet aggregation does not require enhanced thromboxane formation. In healthy volunteers, ticlopidine inhibited ADP-induced α-granule secretion without addition of anticoagulants but did not affect plasma thromboxane A_2 (TXB_2) levels.[67] Thus, there is no relevant effect of thienopyridines on arachidonic acid metabolism in platelet and the vessel wall.[13]

Effects of Thienopyridines in vivo

The significance of ADP for platelet function in vivo is species-dependent and can be assessed by studying the consequences of its inhibition. There is prolongation of bleeding time by intravenous infusion of ADP-removing enzymes in rats and rabbits[11] but no prolonged bleeding after ASA in rats. This suggests that, in the rat, ADP plays a key role in thrombogenesis.[68] In man, both ADP and TXA_2 synergize in the hemostatic process[11] and the relative proportion of either agonist depends on the conditions of stimulation.

ADP-induced platelet secretion and thromboxane formation, if any, are much less pronounced in vivo than in many in vitro experiments, when shear-stress and cell/cell interactions with the vessel wall, white and red cells are absent and the external Ca^{++} concentration is lowered to less than 1% of normal, for example in citrated plasma.[69–70] Thus, in vitro data, generated to understand the mechanisms of action of ADP and thienopyridines, are of great value but not directly relevant to the physiological reality in vivo.

Another aspect to be considered in vivo is the presence of other cells that contain considerable amounts of adenine nucleotides, in particular erythrocytes. Valles et al[71] have shown that human erythrocytes are potent stimulators of platelet function and recruitment, an effect that is also detectable after ASA treatment and is partially due to ADP derived from red cells. As little as 10% non-ASA-treated platelets are sufficient to allow significant enhancement of erythrocyte-induced platelet activation. The significance of ADP for platelet activation was also substantiated by the effects of ADP removal by enzymatic degradation.[72] In the case of endothelial injury, the ADPase activity of the endothelium[73] may be reduced, thus allowing for higher local levels of ADP at a site of platelet adhesion. ATP in whole blood is predominantly or entirely dephosphorylated to ADP.[74] Transfer experiments have additionally shown that ADP may account for part of the platelet

stimulation, originally ascribed to TXA_2.[75] Thus, ADP from platelet and non-platelet sources is an important agonist for platelets in circulating blood,[11,71] in particular at sites of vessel injury. Consequently, agents, such as the thienopyridines, may become most valuable agents to prevent the consequences of ADP accumulation for thrombosis.

It is beyond the scope of this review to discuss in detail the clinical data that have been elaborated using thienopyridines as antithrombotic agents in vivo. Only one type of vessel injury and thrombogenesis that appears to be particularly relevant for ADP, will be discussed: platelet deposition and activation in relation to injury/stenosis-induced regional blood flow reductions.

ADP, Thienopyridines and Cyclic Blood Flow Reductions

Exposure of platelets to shear-stress results in ADP release, even in the absence of detectable platelet lysis. This is also not associated with significant changes in cyclic AMP.[76,77] Shear-stress does not result in increased thromboxane formation but rather 12-HETE generation;[81] the latter has no effect on ADP-induced aggregation.[78] Consequently, this type of platelet activation is only modestly sensitive to ASA.[79,80] Experimental data suggest that platelets from ticlopidine-treated volunteers exhibit reduced aggregation and reduced deposition to the subendothelium under standardized shear-stress conditions in vitro [81] and after desmopressin stimulation ex vivo.[82]

Recent studies have demonstrated that platelet-derived ADP is an important factor for cyclic flow variations in stenosed coronary arteries. Yao et al,[83] using the model of coronary artery stenosis-induced cyclic flow variations in dogs, have shown that clopidogrel prevents the reoccurrence of cyclic flow reductions, being almost equally effective as the combination of apyrase and thromboxane receptor blockade (SQ 29548). The compound did not reduce plasma thromboxane levels. The restoration of flow reduction by adrenaline was markedly inhibited in clopidogrel-treated dogs. Thus, the plasma adrenaline levels necessary to restore flow variations were 20 times higher after apyrase, compared with no treatment, 100 times higher after apyrase in combination with SQ 29548, and more than 5000-fold higher after clopidogrel. This suggested that the combined inhibition of thromboxane and ADP-related mechanisms of platelet activation may provide substantial protection from shear-stress-induced platelet activation at a site of endothelial injury. Similar results were obtained in pigs with injury-stenosis-induced injury of the femoral artery, where clopidogrel was found to be more effective than aspirin.[84] However, neither ticlopidine (i.p. 40 mg/kg for 3 days) nor ASA prevented intimal proliferation of smooth muscle cells in the rabbit after endothelial injury under conditions where reduction of thrombocytopenia or treatment with a platelet-phosphodiesterase inhibitor was effective.[85,86] It is now known that growth factors, such as basic fibroblast derived growth factor (bFDGF) and platelet derived growth factor (PDGF) are important mediators of smooth muscle cell proliferation and mitogenesis. The antiproliferative actions of these compounds are less sensitive to elevation in cAMP. Moreover, platelets are not the only source of their formation. These data agree with negative findings on graft neointima proliferation[87] and hypercholesterolemia-induced intimal thickening.[43] However, there is one study where ticlopidine (50 mg/kg × day) for 1 and 2 months reduced intimal proliferation in balloon-denuded rabbit aorta by 50%.[88]

Ticlopidine Pharmacokinetics—Search for the Active Principle

No thienopyridine exhibits any significant antiplatelet effects in vitro.[4] Thus, the irreversible antiplatelet effects in vivo has either to be explained by postulating a time-dependent inactivation process at the level of the platelet ADP receptor that, like reserpine treatment for biogenic amine depletion, starts immediately but requires several days to result in a functionally significant exhaustion of storage pools. Similar changes may not be obtained in vitro because of the short incubation period and the different experimental approach. Alternatively or additionally, the antiplatelet actions of thienopyridines are not due to the compounds themselves but caused by a yet unidentified metabolite. Clearly, both hypotheses do not exclude each other.

Several lines of evidence suggest the generation of an active thienopyridine metabolite, probably at the intestinal level.[4] There is evidence that an active metabolite is circulating in the plasma. The nature of the biologically relevant metabolite is, however, unknown. None of the 13 isolated stable metabolites of ticlopidine shows significant antiplatelet effects in vitro.[4]

Inhibition of platelet aggregation was obtained after incubation with plasma from ticlopidine-treated subjects.[37] Others were unable to confirm a similar inhibitory effect of treated plasma in rats but showed that significant ADP-antagonistic activity was retained in washed platelets after ticlopidine treatment.[12] Studies in man[89] could also not show any antiplatelet effect of plasma from ticlopidine-treated subjects on platelets of untreated persons. Interestingly, there was a significant amount of (unchanged) ticlopidine in platelets. This was taken as evidence that the platelet compartment represents the pharmacological target of ticlopidine. While this certainly is true, it should also be considered that the maximum platelet concentration of ticlopidine in this study was only about 1% of the maximum plasma level of the compound, i.e. relatively low.

After a nearly complete (80–90%) absorption in

the small intestine, ticlopidine is rapidly metabolized and is subject to extensive first-pass metabolism in the liver. In man, this hepatic metabolism of ticlopidine is age-dependent[90] and may, eventually, interfere with the metabolism of other drugs that are subject to extensive hepatic clearance, such as theophylline.[91] In the case of ticlopidine, this hepatic metabolism involves bioactivation, most convincingly demonstrated by the higher activity of oral than parenteral drug administration.[4]

Alternatively, the bioactivation may occur during enteral absorption and/or within the presystemic circulation. Thus, platelets within the portal and mesenteric circulations are exposed to the full concentration of the inhibitor but not platelets in the systemic circulation after intravenous injection. The parallel to platelet acetylation in the presystemic circulation by ASA is evident.[92]

More detailed information on the relationship between pharmakodynamics and pharmacokinetics is now available for clopidogrel.[93] The compound, in the rat, was found to be equally effective with and without passing the enterohepatic circulation. However, clopidogrel lost all of its antiaggregatory activity after functional hepatectomy by implantation of a porto-jugular shunt, whereas circulation of the substance through an isolated liver preparation resulted in a largely normal antiplatelet effect. These findings are suggestive for bioactivation of thienopyridines in the liver and portal circulation, findings, again similar to ASA,[92] where in a similar model, deacetylation was reduced by introducing a porto-caval shunt.[94]

The Profile of Action of Thienopyridines—Conclusions

The two thienopyridines, ticlopidine and clopidogrel, represent a class of antiplatelet compounds that selectively interfere with ADP-induced platelet stimulation. The compounds or the active principles thereof antagonize the inhibition of platelet adenylyl cyclase by ADP, probably at the receptor and/or G-protein level. Thienopyridines do not directly interfere with any other known platelet stimulatory or inhibitory mechanism. In particular, there is no interference with arachidonate metabolism in the platelet or the vessel wall.

These properties make thienopyridines useful compounds in situations when ADP becomes an important platelet stimulating factor. This appears to be the case in any type of shear-stress-induced platelet activation, including vessel stenoses and local endothelial injuries. Experimental data demonstrate a considerable antithrombotic efficacy which is also confirmed in clinical trials.

The definition of a benefit/risk ratio of thienopyridines in comparison to ASA requires a more detailed analysis of available clinical data on ticlopidine which is beyond the scope of this article. There are also no published data on larger clinical trials with clopidogrel. However, studies are underway and according to current information, ticlopidine may be considered an alternative to ASA, in particular for stroke prevention in patients at enhanced vascular risk and ASA-intolerance or poor ASA-response.

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