Platelet ADP Receptors and their Antagonists

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Abstract: ADP plays a crucial role in haemostasis and thrombosis and its receptors are potential target for antithrombotic drugs.The knowledge of the ADP-receptors has been amplified by recent discoveries. This review highlights the ADP-receptors models and their antagonists described in the recent literature, mainly the thienopyridine derivatives.

KeyWords: Adenosine diphosphate, ADP receptors, P2 receptors, thienopyridine, antithrombotics, platelets.

INTRODUCTION

ADP (adenosine diphosphate) plays a crucial role in haemostasis and thrombosis and its receptors are potential targets for antithrombotic drugs. Transduction of the ADP-induced intracellular signaling events involves the interaction between ADP and P2 receptor. A three-receptor model has recently been proposed in which the P2 receptors are composed of three distinct subtypes.

Actually, two different types of blockers against P2 receptors have been reported : thienopyridine derivatives and ATP analogue inhibitors. Ticlopidine and clopidogrel, both thienopyridine derivatives, are orally active inhibitors of ADP-induced platelet aggregation. Recently, a novel thienopyridine derivative, CS-747, has been reported.

This article focuses on the ADP-receptor model and their antagonists, specially the thienopyridine derivatives.

ADP RECEPTORS THEORIES

ADP (**1**) [Fig. (**1**)] is a platelet activator released from red blood cells, activated platelets, and damaged endothelial cells which induces platelet adhesion and aggregation [1,2].

ADP is considered to be a weak platelet agonist by itself as compared for example to thrombin and collagen [3].

Adenine nucleotides interact with P2 receptors, which are widely distributed in many different cell types including endothelial, smooth muscle, epithelial and blood cells, mastocytes and neurones and regulate a broad range of physiological processes.

These receptors are divided into two main groups : the P2X ligand-gated ion-channel receptors and the P2Y G-protein-coupled receptors.

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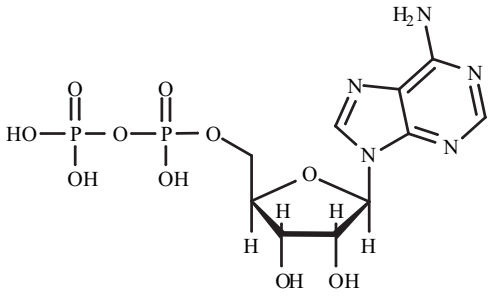
Platelet P2 receptors have been shown to consist of three representatives, namely $P2Y_1$, $P2Y_{12}$ and $P2X_1$ [Fig. (**2**)]. Coactivation of both the $P2Y_1$ and $P2Y_{12}$ receptors is necessary for normal ADP-induced platelet aggregation since inhibition of either receptor is sufficient to block it [4]. The $P2Y_1$ receptor is responsible for intracellular calcium mobilization, shape change and transient aggregation. The $P2Y_{12}$ receptor coupled to adenylyl cyclase inhibition is accountability for amplification of platelet aggregation and the potentiation of platelet secretion. Finally, a $P2X_1$ receptor is responsible for rapid calcium influx; it could synergise with the $P2Y_1$ receptor.



(**1**) Adenosine diphosphate (ADP)

Fig. (1). Chemical structure of adenosine diphosphate (ADP).

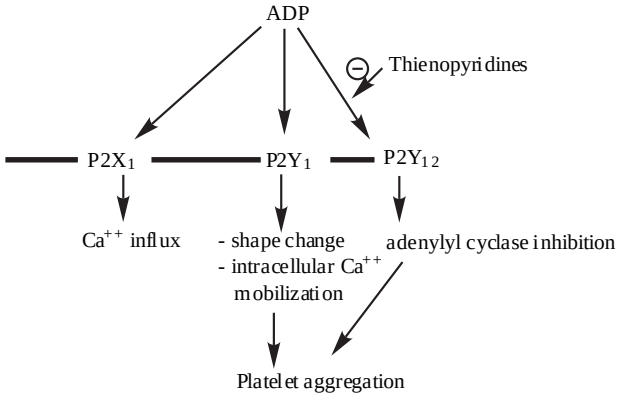


Fig. (2). ADP platelet receptors.

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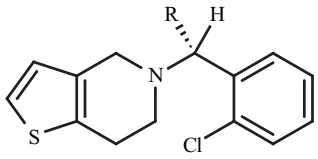
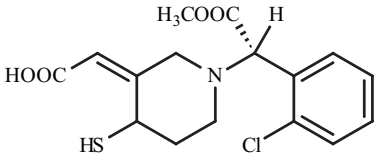
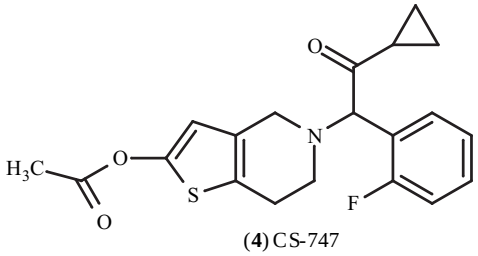


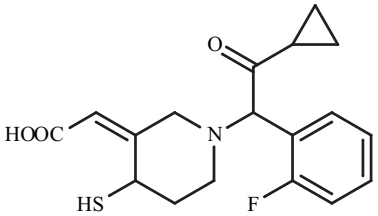
(**2**) ticlopidine: R = H
(**3**) clopidogrel: R = $COOCH_3$



(**3a**) active metabolite of clopidogrel



(**4**) CS-747



(**4A**) active metabolite of CS-747 (R9922)

Fig. (3). Chemical structures of thienopyridine derivatives and metabolites.

ADP ANTAGONISTS

ADP is clearly of physiological and pathological importance in platelet function, as shown by the decreased platelet aggregation and increased resistance to thromboembolism of mice lacking $P2Y_1$ receptors [5,6] or in which the $P2Y_1$ receptors are desensitized by ambiant ADP due to a lack of vascular ATP diphosphohydrolase [7].

The thienopyridines (tetrahydrothienopyridines) [Fig. (**3**)] compounds ticlopidine (**2**) and clopidogrel (**3**) were the first $P2Y_{12}$ inhibitors to be used clinically as antithrombotic drugs [8]. Ticlopidine was discovered in 1972 and marketed in 1978 even though its mechanism of action was not understood. However, its selectivity as an ADP inhibitor was well recognized and helped to elucidate the role of ADP in arterial thrombosis [9-13]. Clopidogrel is a follow-on analog of ticlopidine. It was developed in 1986 and has been on the market since 1997.

They are inactive *in vitro* and have to be metabolised in the liver to acquire their antiaggregatory properties [9,14] [Fig. (**3**)]. The thienopyridines inhibit ADP-induced inhibition of adenylyl cyclase [15], prevent the ADP-induced inhibition of the cytoskeletal associated protein VASP (vasodilatator-stimulated phosphoprotein) phosphorylation, and prevent the association of labeled G proteins with platelet membrane. In contrast, they fail to inhibit ADP-induced platelet shape change or calcium flux of the proteins involved in these processes.

Ticlopidine and clopidogrel induce a dose-dependent reduction in the number of 2-methylthio-ADP binding sites on platelets [16]. However, under conditions where the 2-methylthio-ADP binding sites are maximally reduced (by up to 70% reduction) and ADP-induced inhibition of adenylyl cyclase is completely blocked by thienopyridine treatment, ADP can still promote platelet shape change, rise in intracellular calcium and transient residual aggregation. This occurs through activation of the $P2Y_1$ receptor, which is insentive to thienopyridines [16-19].

Ticlopidine has been reported to reduce the risk of nonfatal myocardial infarction (MI) and vascular death in patients with unstable angina by 46% [20, 21] and to decrease the relative risk of current stroke, MI, or vascular death in patients who suffered from a recent thromboembolic stroke by 30% [22]. Moreover, treatment with ticlopidine has proven to reduce the mortality in patients with intermittent claudication.

This was the clinically most significant result from STIMS, the Swedish Ticlopidine Multicenter Study. Indeed, during an average treatment period of 5.6 years, 153 of the 687 patients died, 26.1% in the placebo group and 18.5% in the ticlopidine group. The incidence of fatal vascular events in the two groups was 12% and 6%, respectively [23].

Ticlopidine has also been reported to be slightly more effective than aspirin in preventing fatal and nonfatal strokes in patients of both sexes with transient ischemic attacks (TIA), amaurosis fugax, or reversible neurologic deficits [24] and to improve the long-term patency of saphenous vein bypass grafts in patients with peripheral vascular disease (PVD) by 19% compared to patients receiving a placebo [25]. Finally, ticlopidine is also frequently used, often concurrently with aspirin, to prevent thrombosis and vascular events in patients who have coronary artery stents implanted [26-28]. Diarrhea, nausea and vomiting are common with ticlopidine since these side-effects occur in 30% to 50% of recipients. Neutropenia is the most serious side-effect reported with ticlopidine and occurs in 2.1% of ticlopidine-treated patients [22]. Bone marrow aplasia and thrombotic thrombocytopenic purpura have also been reported [29-31]. Fortunately, these are usually reversible after drug withdrawal.

Clopidogrel has an antithrombotic activity several times greater than ticlopidine and aspirin. Clopidogrel has a more favourable side-effect profile than ticlopidine, and fatal complications have not been reported. Once again, gastrointestinal problems are the commonest side-effects. Neutropenia was rare and was less frequent in the clopidogrel-treated than the aspirin-treated group. Overall, bleeding time was uncommon, and the frequency of any bleeding event was similar for aspirin and clopidogrel (9.27% vs 9.28%) [32]. Indeed, clopidogrel's efficacy in the

secondary prevention of MI, stroke, and vascular disease in 19,185 patients who had previously suffered a stroke or MI or had peripheral vascular disease was compared to aspirin in the CAPRIE (Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events) trial [33]. In this study, the long-term administration of clopidogrel to patients with atherosclerotic vascular disease was more effective than aspirin in reducing the combined risk of ischaemic stroke, MI, or vascular death and the overall safety profile of clopidogrel was at least as good as that of medium-dose aspirin. Moreover, data from the CLASSICS (Clopidogrel Aspirin Stent International Cooperative Study) trial, in which 1020 patients undergoing stent implantation were randomised to a combination of 75 mg clopidogrel for four weeks (with or without a 800 mg loading dose) and aspirin or ticlopidine and aspirin demonstrated that safety/tolerability of clopidogrel (plus aspirin) was superior to that of ticlopidine (plus aspirin) and that both thienopyridines showed a comparable efficacy with regard to cardiac events after successful stenting [33].

CS-747 (**4**) is a novel antiplatelet agent that generates an active metabolite, R-99224 (**4a**), *in vivo*. CS-747 itself was totally inactive *in vitro*. *In vivo* pharmacological profile of CS-747 after single oral administration to rats were examined.

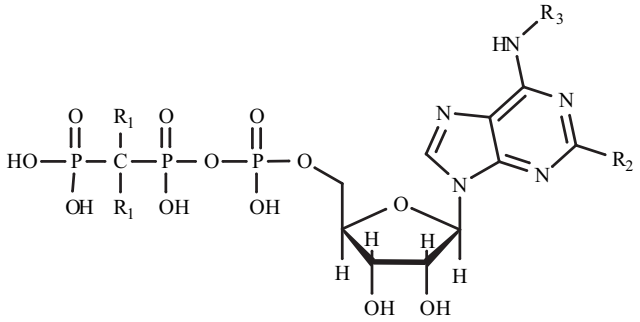
Orally administered CS-747 (0.3 - 10 mg/kg) partially but significantly decreased [^{3}H]-2-methylthio-ADP binding to rat platelets (compared with ADP, 2-methylthio-ADP is 3-5 times as active as an aggregating agent and 150-200 times as active as an inhibitor of cyclic AMP accumulation). CS-747 (3 mg/kg, *per os*) treatment neutralized ADP-induced decreases of cyclic AMP concentrations induced by prostaglandin E_1, suggesting that metabolites of CS-747 interfere with P2Y G-protein-coupled receptors.

CS-747 (0.3 and 3 mg/kg, *per os*) markedly inhibited *ex vivo* washed platelet aggregation in response to ADP but not to thrombin. CS-747 also exhibited a marked inhibition of ADP-induced *ex vivo* platelet aggregation in platelet-rich plasma with a rapid onset (<0.5 h) and long duration (>3 days) of action (ED_{50} at 4 h : 1.2 mg/kg) and prevented thrombus formation in a dose-related manner with an ED_{50} value of 0.68 mg/kg. In this test, CS-747 was more potent than clopidogrel (6.2 mg/kg) and ticlopidine (>300 mg/kg). CS-747, clopidogrel, and ticlopidine prolonged the bleeding time. The order of potency of these agents in this activity was the same as that in antiaggregatory and antithrombotic activities [34].

R-99224 (**4a**) (10 μM) in combination with ARL-66096 (0.3 μM), an ATP analogue-type P2Y G-protein-coupled receptor antagonist, produced no additional inhibition of [^{3}H]-2-MeS-ADP binding. In contrast, [^{3}H]-2-MeS-ADP binding was completely abolished by R-99224 (10 μM) in combination with A3P5PS (300 μM), a selective $P2Y_1$ antagonist, suggesting that R-99224 selectively binds to the P2Y G-protein-coupled receptors.

R-99224 (0.01 - 3 μg/ml) inhibited ADP-induced [^{125}I]-fibrinogen binding to human platelets in a concentration-dependent manner. R-99224 (0.1 - 1 μg/ml) also inhibited the ADP-induced decrease in cyclic AMP levels in PGE_1-stimulated platelets, whereas the agent did not affect ADP (10 μM)-induced Ca^{2+} mobilization. These findings suggest that R-99224 is a selective and irreversible antagonist of P2Y G-protein-coupled receptors and that R-99224 is a responsible molecule for *in vivo* actions of CS-747 [35].

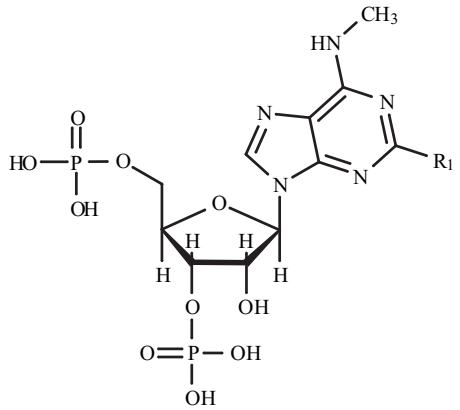
ATP analogues of the AR-C series [Fig.(**4**)] like 2-propylthio-β,γ-difluoromethylene ATP (AR-C66096MX), AR-C67085MX, AR-C69931MX strongly inhibit ADP induced platelet activation *in vitro* without interfering with shape change or calcium mobilisation. Indeed, they interact with $P2Y_{12}$ receptors.



AR-C66096MX : R1 = F, R2 = SPr, R3 = H
AR-C67085MX : R1 = Cl, R2 = SPr = R3 = H
AR-C69931MX : R1 = Cl, R2 = SCH2CH2CF3, R3 = CH2CH2SCH3

Fig. (4). Chemical structures of $P2Y_{12}$ receptor antagonists.

Antagonists of other ADP receptors have been described [Fig.(**5**)]. MRS 2179 (**5a**) has been synthetized and shown an efficient $P2Y_1$ antagonist propriety. However, MRS2179 has also been reported to be an antagonist of the $P2X_1$ receptor *in vitro*. A new compound, MRS2279 (**5b**), seems to be more selective and is a strong antagonist of ADP-induced platelet aggregation [14].



(**5a**) MRS2179 : R1 = H
(**5b**) MRS2279 : R1 = Cl

Fig. (5). Chemical structures of MRS2179 and MRS2279, two $P2Y_1$ receptor antagonists.

CONCLUSION

Forty years ago, ADP was identified as a factor influencing platelet adhesion and inducing platelet aggregation. ADP was rapidly recognised as one of the most

important mediators of haemostasis and thrombosis. During the last five years, the platelet ADP receptors have been identified. Ticlopidine and clopidogrel are part of the standard antiplatelet therapy of vascular diseases. Other ADP modulators such as CS-747 and MRS2279 are currently under evaluation as antithrombotic agents. Further research directions include the signal transduction of the $P2Y_1$ and $P2Y_{12}$ receptors.

REFERENCES

[1] Meyers, K.M.; Holmsen, H.; Seachord, C.l. *Am. J. Physiol.* **1982**, *243*, 454.

[2] Gardner, A.; Jonsen, J.; Laland, S.; Hellem, A.; Owren, P. *Nature* **1961**, *192*, 531.

[3] Siess, W. *Physiol. Rev.* **1989**, *69*, 58.

[4] Jin, J.; Kunapuli, S.P. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 8070.

[5] Fabre, J.E.; Nguyen, M.; Latour, A.; Keifer, J.A.; Audoly, L.P.; Coffman, T.M.; Koller, B.H. *Nat. Med*. **1999,** *5*, 1199.

[6] Léon, C.; Hecheler, B.; Vial, C.; Leray, C.; Ohlmann, P.; Dierich, A.; LeMeur, M.; Cazenave, J.-P.; Gachet, C. *J. Clin. Invest.* **1999**, *104*, 1731.

[7] Enjyoji, K.; Sevigny, J.; Lin, Y.; Frenette, P.S.; Christie, P.D.; Esch, J.S.; Imai, M.; Edelberg, J.M.; Rayburn, H.; Lech, M.; Beeler, D.L.; Csizmadia, E.; Wagner, D.D.; Robson, S.C.; Rosenberg, R.D. *Nat. Med*. **1999**, *5*, 1010.

[8] Savi, P.; Herbert, J.M. *Haematologica* **2000**, *85*, 73.

[9] Quinn, M.J.; Fitzgerald, D.J. *Circulation* **1999**, *100*, 1667.

[10] Feliste, R.; Delebassee, D.; Simon, M.F.; Chap, H.; Defreyn, G.; Vallee, E.; Douste-Blazy, L.; Maffrand, J.P. *Thromb. Res.* **1987,** *48*, 403.

[11] Maffrand, J.P.; Bernat, A.; Delebassee, D.; Defreyn, G.; Cazenave, J.P.; Gordon, J.L. *Thromb. Haemost.* **1998**, *59*, 225.

[12] Defreyn, G.; Bernat, A.; Delebassee, D.; Maffrand, J.P. *Semin. Thromb. Hemost.* **1989**, *15*, 288.

[13] Sharis, P.J.; Cannon, C.P.; Loscalzo, J. *Ann. Intern. Med.* **1998**, *129*, 394.

[14] Gachet, C. *Thromb Haemost.* **2001,** *86*, 222.

[15] Defreyn, G.; Gachet, C.; Savi, P.; Driot, F.; Cazenave, J.P.; Maffrand, J.P. *Thromb. Haemost.* **1991,** *65*, 186.

[16] Savi, P.; Laplace, M.C.L.; Maffrand, J.P.; Hebert, J.M. *J. Pharmacol. Exp. Ther.* **1994**, *269*, 772.

[17] Savi, P.; Pereillo, J.M.; Uzabiaga, M.F.; Combalbert, J.; Piccard, C.; Maffrand, J.P.; Pascal, M.; Hebert, J.M. *Thromb. Haemost.* **2000**, *84*, 891.

[18] Hollopeter, G.; Jantzen, H.M.; Vincent, D.; Li, G.; England, L.; Ramakrishnan, V.; Yang, R.B.; Nurden, P.; Nurden, A.; Julius, D.; Conley, P.B. *Nature* **2001**, *409*, 202.

[19] Leon, C.; Gachet, C.; Ohlmann, P.; Hechler, B.; Cazenave, J.P.; Lecchi, A.; Cattaneo, M. *Thromb. Haemost.* **1999**, *81*, 775.

[20] Bennett, J.S. *Annu. Rev. Med.* **2001**, *52*, 161.

[21] Balsano, F.; Rizzon, P.; Violi, F.; Scurtinio, D.; Cimminiello, C.; Aguglia, F.; Pasotti, C.; Rudelli, G. *Circulation* **1990**, *82*, 17.

[22] Gent, M.; Blakely, J.A.; Easton, J.D.; Ellis, D.J.; Hachinski, V.C.; Harbison, J.W.; Panak, E.; Roberts, R.S.; Sicurella, J.; Turpie, A.G. *Lancet* **1989**, *1*, 1215.

[23] Janzon, L. *Vasc. Med.* **1996**, *1*, 141.

[24] Hass, W.K.; Easton, J.D.; Adams, H.P.; Pryse-Phillips, W.; Molony, B.A.; Anderson, S.; Kamm, B. *N. Engl. J. Med.* **1989**, *321*, 501.

[25] Becquemin, J.P. *N. Engl. J. Med.* **1997**, *337*, 1726.

[26] Schoming, A.; Neumann, F.J.; Kastrati, A.; Schuhlen, H.; Blasini, R.; Hadamitzky, M.; Walter, H.; Zitzmann-Roth, E.M.; Richardt, G.; Alt, E.; Schmitt, C.; Ulm, K. *N. Engl. J. Med.* **1996**, *334*, 1084.

[27] Leon, M.B.; Baim, D.S.; Popma, J.J.; Gordon, P.C.; Cutlip, D.E.; Ho, K.K.; Giambartolomei, A.; Diver, D.J.; Lasorda, D.M.; Williams, D.O.; Pocock, S.J.; Kuntz, R.E. *N. Engl. J. Med.* **1998**, *339*, 1665.

[28] Albiero, R.; Hall, P.; Itoh, A.; Blengino, S.; Nakamura, S.; Martini, G.; Ferraro, M.; Colombo, A. *Circulation* **1997**, *95*, 1145.

[29] Benett, C.; Weinberg, P.; Rozenberg-Ben-Dror, K.; Yarnold, P.; Kwaan, H.; Green, D. *Ann. Intern. Med.* **1998**, *128*, 541.

[30] Kupfer, Y.; Tessler, S. *N. Engl. J. Med.* **1997**, *337*, 1245.

[31] Page, Y.; Tardy, B.; Zeni, F.; Comtet, C.; Terrana, R.; Bertrand, J.C. *Lancet* **1991**, *337*, 774.

[32] CAPRIE Steering Committee. *Lancet* **1996**, *348*, 1329.

[33] Bertrand, M.E.; Rupprecht, H.J.; Urban, P.; Gershlick, A.H. *Circulation* **2000**, *102*, 624.

[34] Sugidachi, A.; Asai, F.; Ogawa, T.; Inoue, T.; Koike, H. *Br. J. Pharmacol.* **2000**, *129*, 1439.

[35] Sugidachi, A.; Asai, F.; Yoneda, K.; Iwamura, R.; Ogawa, T.; Otsuguro, K.; Koike, H. *Br. J. Pharmacol.* **2001**, *132*, 47.