editorial

The platelet ADP receptors

MARCO CATTANEO, CHRISTIAN GACHET*
Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Ospedale Maggiore, University of Milan, Italy *INSERM U.311. Biologie et Pharmacologie de l'Hémostase et de la Thrombose, Etablissement Francais de Sang-Alsace, Strasbourg, France

haematologica 2001; 86:346-348
http://www.haematologica.it/2001_04/0346.htm

Correspondence: Marco Cattaneo, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Ospedale Maggiore, University of Milan, Italy. E-mail: marco.cattaneo@unimi.it

Adenosine-5'-diphosphate (ADP) was recognized as an inducer of platelet aggregation in the early sixties.[1,2] Although itself a weak platelet agonist, ADP plays a key role in platelet function because, when it is secreted from the platelet dense granules where it is stored, it amplifies the platelet responses induced by other platelet agonists.[3] The amplifying effect of ADP on platelet aggregation may account for the critical role played by ADP in hemostasis and in the pathogenesis of arterial thrombosis, which is documented by a number of observations: 1) pharmacologic inhibition of ADP-induced platelet aggregation decreases the risk of arterial thrombosis;[4] 2) patients lacking releasable ADP in granule stores or with congenital abnormalities of the platelet ADP receptors have a bleeding diathesis;[5-7] 3) CD39, the endothelial cell ecto-ADPase, is a critical component in the regulation of thrombogenesis.[8,9]

Biochemical, pharmacologic and clinical studies led to the proposal of a model of three purinergic receptors contributing separately to the complex process of ADP-induced platelet aggregation: the $P2X_1$ ionotropic receptor responsible for rapid influx of ionized calcium into the cytosol, the $P2Y_1$ metabotropic receptor responsible for mobilization of ionized calcium from internal stores which initiates aggregation, and the $P2Y_{12}$ receptor coupled to adenylyl cyclase inhibition (previuosly named in different ways by different authors: P2cyc, $P2Y_{ADP}$, $P2T_{AC}$), which is essential for the full platelet aggregation response to ADP, although no causal relationship exists between adenylyl cyclase inhibition and platelet aggregation.[3,10-13] This receptor is the molecular target of the ADP-selective antiaggregating drugs, ticlopidine and clopidogrel. In addition, it is defective in patients with a bleeding diathesis that is characterized by selective impairment of platelet responses to ADP.[6,7,14,15] On the other hand, studies with $P2Y_1$ receptor-deficient mice clearly demonstrated the critical role of this receptor in hemostasis and in thrombosis.[16,17] Despite the recent progresses in the understanding of the mechanisms involved in ADP-induced platelet responses, many issues are still unknown or remain controversial: 1) the role of the $P2X_1$ receptor in platelet function; 2) the nature of the effector(s) in the Gi pathway of ADP-induced platelet aggregation, to name but a few.

In consideration of the very important recent achievements and the rapid evolution of this area of research we thought that time was ripe for organizing a meeting in which some of the most distinguished scientists in this research field could gather to exchange their experiences and to clarify the state of the art and future perspectives. The meeting took place in March, 2000 in La Thuile, a small village in the Italian Alps, which provided a beautiful frame to what proved to be a very stimulating and interesting scientific meeting.

There were several lectures and oral communications,[18-46] whose contents may be found on Internet at http://www.haematologica.it/e-page.html. The following conclusions were achieved.

Well-established points

The model of three purinergic receptors mediating all the effects of ADP on platelets, namely the ligand-gated non-selective cation channel $P2X_1$ responsible for a rapid calcium entry, the $P2Y_1$ receptor, coupled to Gq, responsible for calcium mobilization, shape change and initiation of platelet aggregation by ADP and the P2Y receptor negatively coupled to adenylyl cyclase ($P2Y_{12}$), responsible for amplification and completion of the platelet response to ADP is now well established and agreed by all the investigators working in the field. Also well established, albeit less well known, are the methodologic problems in the study of platelet responses to ADP. A special homage was rendered to J. Fraser Mustard who defined factors influencing ADP-induced platelet aggregation. The role of external ionized calcium as well as of albumin in the suspensions of washed platelets, the quality of the blood samples, the choice of anticoagulants, and comparison between species, among other aspects, were discussed. Finally, there is consensus concerning the structures of the cloned P2Y receptors and the pharmacology of 5 of them: P2Y1, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$. The pharmacologic properties of the so-called AR-C compounds as well as of the thienopyridine compounds, selective antagonists and inhibitors of $P2Y_{12}$ are also clearly accepted by all although some controversies remained in terms of comparison of the two classes of drugs. Finally, congenital disorders of platelet function, among which the selective defect of ADP-induced platelet aggregation related to a $P2Y_{12}$ defect, were extensively reviewed.

New data

The following new data were presented:

1) ADP is an important cofactor in phosphatidylinositol 3-kinase (PI-3K) activation both in the stabilization of TRAP-induced platelet aggregation and in FcγRIIa-induced platelet activation.
2) Gai2 deficiency results in partial impairment of ADP-induced platelet activation, confirming a role for Gai2

in ADP signaling.

3) The Gi pathway is a necessary complementary signal in platelet aggregation, independently of the starting stimulus (PKC or PLC).
4) In Gaq knockout mice, ADP can restore collagen-induced platelet aggregation and, at very high concentrations (100 µM), promotes partial aggregation in the absence of calcium signaling and shape change. Similarities of the Gaq deficient mice with the $P2Y_1$ receptor knockout mice were underlined.
5) The $P2Y_{12}$ receptor plays important roles in the potentiation of platelet dense granule secretion and in the exposure of phosphatidylserine and thus, probably in thrombin generation. All these points unravel the molecular mechanisms underlying the crucial role of ADP as a cofactor in all aspects of platelet activation and emphasize the involvement of the $P2Y_{12}$ receptor in these processes. The effects of the new AR-C compound, AR-C69931MX, a selective $P2Y_{12}$ receptor antagonist, globally confirm these findings since it was widely used either as a tool or as a drug both *in vitro* and *in vivo*.
6) $P2Y_1$ knockout mice are resistant to a thrombin dependent-thromboembolism model. Moreover, *in vivo* pharmacologic modulation of the $P2Y_1$ receptor with MRS2179 results in a similar resistance to acute thrombosis induced either by collagen-adrenalin or by tissue factor. Thus, the $P2Y_1$ receptor is a promising target for new antiplatelet agents. The regulation of its gene expression by thrombopoietin was also reported.
7) The well known refractory state of platelets to ADP results entirely from the selective desensitization of the $P2Y_1$ receptor probably by internalization while the $P2Y_{12}$ receptor is still functional and responsive to ADP. A role for ADP in modulating platelet adhesion and limiting the expansion of the thrombus was also shown.
8) Recombinant CD39, the ectoATPDase or apyrase-like ectoenzyme, is active both *in vitro* and *in vivo* as an antiplatelet agent, and seems to be a potent and promising antithrombotic drug in stroke.

Controversies

New but controversial were three reports dealing with a possible role of the $P2X_1$ receptor in platelet activation and in hemostasis. The case of a patient with a bleeding disorder that might be due to the presence of a mutated form of the $P2X_1$ receptor was described. The reasons for the discrepancy between the severity of the bleeding diathesis and the mild inhibition of platelet agregation and calcium signals reported were unclear. Two studies reporting on functional properties of the $P2X_1$ receptor, one on shape change induced by a selective $P2X_1$ agonist, one on activation of ERK/MAP kinase through the $P2X_1$ receptor, were extensively discussed and left some key questions unanswered. Further studies are certainly required to unravel the role of this receptor in platelet physiology.

Perspectives

It was planned to organize a second ADP meeting in two years. The hope is the following questions will be answered by then: what is the role of the $P2X_1$ receptor in platelet activation, hemostasis and thrombosis? The availability of $P2X_1$ knockout mice would certainly be of great help, also in consideration of the lack of appropriate selective agonists.

In terms of pharmacology, the use of new drugs selective for ADP, $P2Y_1$ or $P2Y_{12}$ antagonists as well as recombinant CD39 should be better characterized both in animal models and in clinical studies.

References

1. Gaarder A, Jonsen J, Laland S, Hellem A, Owren PA. Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. Nature 1961; 192:531-2.
2. Gaarder A, Laland S. Hypothesis for the aggregation of platelets by nucleotides. Nature 1964; 202:909-11.
3. Cattaneo M, Gachet C. ADP receptors and clinical bleeding disorders. Arterioscler Thromb Vasc Biol 1999; 19: 2281-5.
4. Quinn MJ, Fitzgerald DJ. Ticlopidine and clopidogrel. Circulation 1999; 100:1667-72.
5. Bennett JS. Hereditary disorders of platelet function. In: Hoffman R, Benz EJ Jr, Shattil SS, Furie B, Cohen HJ, Silberstein LE, McGlave P, eds. Hematology. Basic Principles and Practice. New York, NY: Churchill Livingstone; 2000; 2154-72.
6. Cattaneo M, Lecchi A, Randi AM, McGregor JL, Mannucci PM. Identification of a new congenital defect of platelet function characterized by severe impairment of platelet responses to adenosine diphosphate. Blood 1992; 80: 2787-96.
7. Nurden P, Savi P, Heilmann E, et al. An inherited bleeding disorder linked to a defective interaction between ADP and its receptor on platelets. J Clin Invest 1995; 95:1612-22.
8. Marcus AJ, Broekman MJ, Drosopoulos JHF, et al. The endothelial cell ecto-APDase responsible for inhibition of platelet function is CD39. J Clin Invest 1997; 99: 1351-60.
9. Enjyoji K, Sévigny J, Lin Y, et al. Targeted disruption of CD39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nat Med 1999; 9:1010-7.
10. Daniel JL, Dangelmaier CA, Jin J, Ashby B, Smith JB, Kunapuli SP. Molecular basis for ADP-induced platelet activation. I. Evidence for three distinct ADP receptors on human platelets. J Biol Chem 1998; 273: 2024-9.
11. Hechler B, Léon C, Vial C, et al. The P2Y1 receptor is necessary for ADP-induced platelet aggregation. Blood 1998; 92:152-9.
12. Geiger J, Honig-Liedl, Schanzenbacher P, Walter U. Ligand specificity and ticlopidine effects distinguish three human platelet ADP receptors. Eur J Pharmacol 1998; 351:235-46.
13. Jin J, Kunapuli SP. Coactivation of two different G protein-coupled receptors is essential for ADP-induced platelet aggregation. Proc Natl Acad Sci USA 1998; 95:8070-4.
14. Hechler B, Eckly A, Ohlmann P, Cazenave JP, Gachet C. The P2Y1 receptor, necessary but not sufficient to support full ADP-induced platelet aggregation, is not the target of the drug clopidogrel. Br J Haematol 1998; 103: 858-66.
15. Léon C, Vial C, Gachet C, et al. The P2Y1 receptor is normal in a patient presenting a severe deficiency of ADP-induced platelet aggregation. Further evidence for a distinct P2 receptor responsible for adenylyl cyclase inhibition. Thromb Haemost 1999; 81:775-81.
16. Fabre JE, Nguyen M, Latour A, et al. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice. Nat Med 1999; 5:1199.
17. Léon C, Hechler B, Freund M, et al. Defective platelet aggregation and increased resistance to thrombosis in purinergic P2Y1 receptor null mice. J Clin Invest 1999; 104:1731.

18. de Gaetano G. Historical overview of the role of platelets in hemostasis and thrombosis. Haematologica 2000; 85(the Platelet ADP Receptors supplement):3-10.
19. Mills DCB. Historical overview of the role of adp in platelet function. Haematologica 2000; 85(the Platelet ADP Receptors supplement):11-14.
20. Boeynaems J-M, Communi D, Suarez-Huerta N, Janssens R, Robaye B. P2Y receptors. Haematologica 2000; 85(the Platelet ADP Receptors supplement):15-21.
21. Geiger J. Ligand specificIty, regulation and cross-talk of human platelet ADP receptors. Haematologica 2000; 85(the Platelet ADP Receptors supplement):22-26.
22. Kunapuli SP. Interplay of P2 receptor subtypes in platelet function. Haematologica 2000; 85(the Platelet ADP Receptors supplement):27-31.
23. Payrastre B, Gratacap M-P, Trumel C, et al. ADP: an important cofactor of pi 3-kinase activation in human blood platelets. Haematologica 2000; 85(the Platelet ADP Receptors supplement):32-36.
24. Clemetson KJ, Clemetson JM. Congenital disorders of platelet function. Haematologica 2000; 85(the Platelet ADP Receptors supplement):37-45.
25. Nurden P, Gauthier B, Poujol C, Pasquet J-M, Nurden AT. Congenital defects of ADP receptors on platelets Haematologica 2000; 85(the Platelet ADP Receptors supplement): 46-52.
26. Marcus AJ, Broekman JM, Drosopoulos JHF, et al. Human ecto-ADPase/CD39: thromboregulation via a novel pathway. Haematologica 2000; 85(the Platelet ADP Receptors supplement):53-57.
27. Hourani SMO. Pharmacology of the platelet ADP receptors: agonists and antagonists. Haematologica 2000; 85(the Platelet ADP Receptors supplement):58-65.
28. Humphries RG. Pharmacology of ar-c69931mx and related compounds: from pharmacological tools to clinical trials. Haematologica 2000; 85(the Platelet ADP Receptors supplement):66-72.
29. Savi P, Herbert JM. Pharmacology of Ticlopidine and Clopidogrel. Haematologica 2000; 85(the Platelet ADP Receptors supplement):73-77.
30. Violi F, Di Lecce VN, Loffredo L. Clinical trials with ADP receptor antagonists. Haematologica 2000; 85(the Platelet ADP Receptors supplement):78-80.
31. Jantzen HM, Milstone DS, Gousset L, Conley PB, Mortensen R. Impaired platelet activation in gai2-deficient mice. Haematologica 2000; 85(the Platelet ADP Receptors supplement):85.
32. Pulcinelli FM, Di Santo S, Coletti V, Pignatelli P, Riondino S. Independent activation of protein kinase C or phospholipase C can induce platelet aggregation provided a gi protein-coupled receptor is activated. Haematologica 2000; 85(the Platelet ADP Receptors supplement):85.
33. Ohlmann P, Eckly A, Freund M, et al. ADP induces partial platelet aggregation without shape change and potentiates collagen induced aggregation in the absence of Gaq Haematologica 2000; 85(the Platelet ADP Receptors supplement):86.
34. Cattaneo M, Lecchi A. ADP potentiates platelet dense granule secretion induced by U46619 or trap through its interaction with the P2cyc receptor. Haematologica 2000; 85(the Platelet ADP Receptors supplement):86.
35. Baurand A, Eckly A, Bari N, et al. desensitization of the platelet aggregation response to ADP: differential down-regulation of the P2Y1 and P2cyc receptors. Haematologica 2000; 85(the Platelet ADP Receptors supplement):87.
36. Varon D, Shenkman B, Tamarin I, Dardik R, Frojmovic M. Transient adhesion refractoriness of platelets under flow conditions: the role of partial activation and microaggregate formation by suboptimal ADP concentration. Haematologica 2000; 85(the Platelet ADP Receptors supplement):87.
37. Tomlinson W, Kirk IP, Humphries RG, Leff P. P2T receptor activation by adp: a permissive role in aggregation of human washed platelets induced by PAF or U46619. Haematologica 2000; 85(the Platelet ADP Receptors supplement):87.
38. Storey RF, Sanderson HM, White AE, May JA, Newby LJ, Heptinstall S. The central role of the p2T receptor in amplification of platelet aggregation, secretion and procoagulant activity. Haematologica 2000; 85(the Platelet ADP Receptors supplement):88.
39. Oury C, Toth-Zsamboki E, Van Geet C, et al. Dominant negative mutation in the platelet P2X1 adp receptor causes a severe bleeding disorder. Haematologica 2000; 85(the Platelet ADP Receptors supplement):89.
40. Rolf MG, Mahaut-Smith MP. Functional roles of P2x1 purinoceptors in human platelets. Haematologica 2000; 85(the Platelet ADP Receptors supplement):89.
41. Oury C, Toth-Zsamboki E, Thys C, Vermylen J, Hoylaerts M. ADP-induced activation of the extracellular-regulated kinase/mitogen-activated protein kinase pathway via the ionotropic P2x1 receptor in platelets. Haematologica 2000; 85(the Platelet ADP Receptors supplement):89.
42. Léon C, Freund M, Ravanat C, Cassel D, Cazenave JP, Gachet C. Tissue factor-induced acute thromboembolism is reduced in P2x1 -knockout mice. Haematologica 2000; 85(the Platelet ADP Receptors supplement):90.
43. Baurand A, Freund M, Cassel D, Cazenave JP. N6-methyl 2'-deoxyadenosine 3'-5'-bisphosphate, a potent and selective P2x1 antagonist, inhibits adp-induced platelet aggregation in vitro and ex vivo and prolongs the bleeding time. Haematologica 2000; 85(the Platelet ADP Receptors supplement):90.
44. Humphries RG, Nicol AK, Tomlinson W, Robertson MJ, Ingall AH, Leff P. Effect of the novel P2t receptor antagonist, AR-C69931MX, on thrombosis and hemostasis in the dog: comparison with GPIIb/IIIa antagonists. Haematologica 2000; 85(the Platelet ADP Receptors supplement):91.
45. Storey RF, Henderson RA, Wilcox RG, Heptinstall S. Superior antiplatelet effects of ar-c69931mx compared to clopidogrel in patients with ischemic heart disease. Haematologica 2000; 85(the Platelet ADP Receptors supplement):92.
46. Jarvis GE, Nassim MA, Humphries RG, Kirk IP. Clopidogrel produces incomplete inhibition of [33P]-2MeSADP binding to human platelets and less inhibition of adp-induced platelet aggregation than the P2T antagonist AR-C69931MX. Haematologica 2000; 85(the Platelet ADP Receptors supplement):92.

Thalidomide in the treatment of multiple myeloma

In this issue, three full papers and one Irreplaceable Image article illustrate the impressive effect of thalidomide in patients with multiple myeloma who have failed conventional therapy. This clearly expands the therapeutic options for this condition.[1,2]

References

1. San Miguel JF, Bladé Creixenti J, García Sanz R. Treatment of multiple myeloma. Haematologica 1999; 84:36-58.
2. Harousseau JL. Optimizing peripheral blood progenitor cell autologous transplantation in multiple myeloma. Haematologica 1999; 84:548-53.