A denosine-5'-diphosphate (ADP) was recognized as an inducer of platelet aggregation in the early sixties. 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. 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; 2) patients lacking releasable ADP in granule stores or with congenital abnormalities of the platelet ADP receptors have a bleeding diathesis; 3) CD39, the endothelial cell ecto-ADPase, is a critical component in the regulation of thrombogenesis.

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 P2X1 ionotropic receptor responsible for rapid influx of ionized calcium into the cytosol, the P2Y1 metabotropic receptor responsible for mobilization of ionized calcium from internal stores which initiates aggregation, and the P2Y12 receptor coupled to adenyl cyclase inhibition (previously named 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, 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 P2X1, responsible for a rapid calcium entry, the P2Y1 receptor, coupled to Gq, responsible for calcium mobilization, shape change and initiation of platelet aggregation by ADP and the P2Y12 receptor negatively coupled to adenylyl cyclase (P2Y12), 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, P2Y2, P2Y4, P2Y6 and P2Y11. The pharmacologic properties of the so-called AR-C compounds as well as of the thienopyridine compounds, selective antagonists and inhibitors of P2Y12, 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 P2Y12 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 P2Y1 receptor knockout mice were underlined.

5) The P2Y1 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 P2Y1 receptor in these processes. The effects of the new AR-C compound, AR-C69931MX, a selective P2Y12 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) P2Y1 knockout mice are resistant to a thrombin dependent-thromboembolism model. Moreover, in vivo pharmacologic modulation of the P2Y1 receptor with MRS2179 results in a similar resistance to acute thrombosis induced either by collagen-adrenal or by tissue factor. Thus, the P2Y1 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 P2Y1 receptor by internalization while the P2Y12 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 P2X1 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 P2X1 receptor was described. The reasons for the discrepancy between the severity of the bleeding diathesis and the mild inhibition of platelet aggregation and calcium signals reported were unclear. Two studies reporting on functional properties of the P2X1 receptor, one on shape change induced by a selective P2X1 agonist, one on activation of ERK/MAP kinase through the P2X1 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 P2X1 receptor in platelet activation, hemostasis and thrombosis? The availability of P2X1 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, P2Y1, or P2Y12 antagonists as well as recombinant CD39 should be better characterized both in animal models and in clinical studies.

References


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):92-96.


References


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.