Cell-Cell Interactions in Thrombosis: Modulation of Platelet Function and Possibilities of Pharmacological Control with Aspirin

Mª Teresa SANTOS, Juana VALLÉS, Justo AZNAR

Department of Clinical Pathology and Research Center, La Fe University Hospital, Valencia, SPAIN

Turk J Haematol 2002;19(2):103-111

Thrombosis is a complex and dynamic phenomenon, involving interaction of endothelial and blood cells, plasma clotting, fibrinolysis and hemorrheologic factors. Platelets play an essential role in the process of thrombosis[1]. Interaction of platelets with collagen and/or thrombin initiates a complex biochemical sequence of signal transmission resulting in a functional cell response.

There are several signal mechanisms involved in platelet activation, including specific receptors, G proteins, phosphatidyl inositol metabolism, synthesis of thromboxane A_2 (TXA_2) and other eicosanoids, changes in cytosolic calcium, and protein phosphorylation, in serine/threonine and tyrosine residues[2]. These signal mechanisms result in a conformational change in the GP IIb IIIa receptor, platelet secretion, platelet–platelet aggregation, exposure to procoagulant phospholipids in the membrane, clot retraction, and synthesis of different metabolic products.

We have found that the products released by activated platelets have a proaggregating action on the target platelets, favoring platelet recruitment and thrombus growth[3-5]. To study these processes, we have developed an experimental procedure that allows activation and platelet recruitment to be assessed separately[4-6].

Platelets, leukocytes and erythrocytes appear in the thrombus[7]. This might indicate that the interaction of platelets with other blood cells is important in regulation of the process of thrombosis. Platelet–leukocyte interaction inhibits platelet reactivity, whereas erythrocyte–platelet interaction enhances it[3-6]. It has also been shown that this cell interaction alters the antithrombocytic effect of aspirin and dipyridamole[4-15].

A well-documented example of this intercellular interaction is the transcellular metabolism of eicosanoids, which occurs among different blood cells, and between blood and endothelial cells[16,17]. In this process, arachidonic acid, compounds of their intermediate metabolism or the specific final products of the metabolism of one cell are changed by another one into specific products of the second, or into new compounds which neither cell can synthesize in an independent manner[16]. Metabolic cooperation in the synthesis of

lipid mediators occurs through various cellular interactions, including interactivus between platelets and endothelial cells, platelets and neutrophils, platelets and erythrocytes, endothelial cells and neutrophils, and neutrophils and erythrocytes[18-23].

Platelet–neutrophil interaction: Experimental studies suggest that polymorphonuclear neutrophils (PMNs) play a role in thrombosis[24]. It is thought that PMNs play a role in vascular disease through their capacity to adhere to endothelial cells and aggregate during acute inflammatory processes[25,26]. Activated PMNs release into the micro environment azurophillic granules, rich in proteases, and oxygen radicals, which may damage endothelial cells, disrupting their thromboregulating function[27-29]. Moreover, PMNs participate in thrombotic events by contributing to coagulant processes and by modulating different stages of the process of thrombosis[30].

There are conflicting reports on the role of PMNs in the process of thrombosis[31]. Some authors report that PMNs stimulate platelet activation, whereas others report inhibition by PMNs[6,12,32-38]. These conflicting results may arise from differences in experimental conditions between studies, in particular whether PMNs are studied at rest or after stimulation and whether whole cells or isolated compounds derived from stimulated leukocytes, such as the cathepsin G or oxidant products, are assessed. Various mechanisms by which PMNs might inhibit platelet function have been suggested: Synthesizing nitric oxide (NO), modulating synthesis of eicosanoids, modifying glycoproteins of the platelet membrane, generating H_2O_2 and other oxidants, membrane ADP-arg, or other mechanisms[6,12,34-42]. Physical proximity between cells is favored by P-selectin, an adhesion protein exposed on the membrane of activated platelets, which binds to a specific receptor in the PMNs, PSGP-1, and between the GP IIb IIIa on platelets and $ß_2$ integrin on PMNs[33,43,44].

Aspirin amplifies the inhibiting effect of PMNs on thrombocytic reactivity, although it does not change the capacity of the platelets to expose P-selectin[6,12,13,37,45-47]. Thus, aspirin may not modify other processes depending on P-selectin that are important in the PMN-platelet interaction, such as the recruitment of PMNs by activated platelets to the forming thrombus in the area of vascular damage, or fibrin formation mediated by platelet P-selectin leading to the expression of tissue factor in leukocytes[30,44,48].

Role of erythrocytes in hemostasis and thrombosis: Erythrocytes (RBCs) also play a role in thrombogenesis[10,49]. This was first reported by Duke in 1910, who found that the prolonged bleeding times in anemic patients became normal after the hematocrit was increased[50]. This clinical association between bleeding times and the hematocrit were confirmed by other authors[51-54]. Erythrocytes have also been involved in atherothrombotic processes and associated with established risk factors such as diabetes, hypercholesterolemia, and hypertension[51,55-63].

Erythrocytes may participate in thrombus formation, changing the hemorrheologic properties of blood, modulating the antiaggregating action of the blood vessels, inactivating prostacyclin, eliminating NO (EDRF) and influencing thrombocytic function, although it has no effect on the ecto-ADP loops of the vascular endothelium[64-72].

Effect of the erythrocytes on thrombocytic reactivity: Erythrocytes act on platelets by physical and biochemical mechanisms[73,74]. Hellem and Gaarder reported for the first time in 1961 on the capacity of erythrocytes to increase thrombocytic function; in this study, platelet adhesiveness to glass increased in the presence of erythrocytes[75]. These authors suggested that ADP released by lysis or cellular microlysis of erythrocytes could be the biochemical factor responsible for increased adhesion-aggregation of platelets in the presence of erythrocytes[76]. It was later shown that erythrocytes increase platelet adhesion and thrombus formation in damaged endothelium[77]. They correct adhesion defects in patients with “storage pool deficiency (SPD)” depending on the hematocrit, they increase spontaneous platelet aggregation, enhancing platelet aggregation due to collagen, they increase platelet activation and recruitment and they shorten the formation time of the thrombus in the platelet function analyzer PFA-100[4,5,14,63,78-82].

The biochemical mechanisms that regulate the effects of erythrocytes on platelet function are not well known. We have shown that erythrocyte–platelet interaction increases in the presence of cellular stimulation, both by ADP concentration (7 times) and ATP (5 times); this occurs without erythrocyte lysis[5]. It is

worth mentioning that the potentiating effect of erythrocytes on the erythrocyte reactivity requires the simultaneous presence of activated platelets and intact erythrocytes[4,5]. The prothrombotic effect of erythrocytes does not occur in the presence of platelet stimulation or erythrocyte lysis. This suggests that, under experimental conditions, the effect of erythrocytes is related more to metabolic than physical factors, requiring the cellular integrity of the erythrocyte.

Erythrocyte–platelet interaction increases hydrolysis of the arachidonic acid of the phospholipids, as well as the metabolism of the platelet enzymes cyclooxygenase and lipooxygenase[4]. This interaction increases the release of eicosanoids and arachidonic acid to the cellular microenvironment, which could stimulate other platelets and provide the substratum needed for the transcellular metabolism with other blood cells. An example of this effect may be how the PMNs use the arachidonic acid in a thrombus formation to generate LTB_4 from the arachidonic acid released by the platelets[83].

Erythrocyte–platelet interaction also leads to a significant increase in release of TXA_2 and 12-HETE and generates proteolytic activity, which may participate in the erythrocyte effect on platelet activation[4,84,85]. This interaction also releases free radicals and iron, which may favor increased platelet activation[86,87]. More recently, we have shown that erythrocytes increase the activation of platelet glycoprotein IIb IIIa, playing a regulating role in the intracellular biochemical mechanisms of platelet signaling[88,89]. Nevertheless, our knowledge of platelet-erythrocyte interactions remains incomplete, since not only do erythrocytes modify the biochemical and functional responses of platelets, but products released by platelets, such as PGE_2 or serotonin, may induce changes in erythrocyte metabolism, fragility and deformability[90-93].

Erythrocyte–platelet interaction: Effect of aspirin: In high-risk patients, cardiovascular or neurological thrombotic episodes may be favored by increased platelet activation in areas of endothelial damage or plaque rupture[94]. Thus, inhibition of platelet function is important in the prophylaxis and treatment of ischemic and thrombotic processes in these patients. The drug of choice in these cases, aspirin, reduces morbidity due to vascular causes in about 15% of patients, and new nonfatal accidents in 30% of patients[95]. Treatment with aspirin is not protective in a high proportion of patients, however, and new therapies are needed to reduce thrombotic risk in these patients.

Aspirin inhibits synthesis of TXA_2 in an irreversible manner[96]. Thrombotic events in patients treated with aspirin could be due to biochemical mechanisms of platelets that surpass the aspirin effect. This might occur because of exposure of platelets to high concentrations of collagen or thrombin, or possibly ADP. Under these conditions, platelets do not require the amplification action of thromboxane to produce a proaggregation response, i.e., platelet activation is stimulated by cyclooxygenase 1 (COX-1) independent mechanisms[97,98].

We have found experimental evidence that aspirin not only acts on platelets by inhibiting COX-1, but also exerts a direct effect on erythrocytes, reducing their prothrombotic potential under certain conditions[5,14]. This newly identified antithrombotic effect of aspirin occurs when platelets of patients treated with aspirin (500 mg) are stimulated with low doses of collagen (0.5-1 µg/mL). Under these conditions, the platelets alone are completely inhibited and the erythrocytes of the same donor, also treated with aspirin, produce no increase in platelet stimulation. Conversely, erythrocytes obtained from the same patients before aspirin administration can significantly stimulate the aspirinized-platelets[5]. Thus, it seems reasonable to suggest that a greater beneficial clinical effect could be achieved by blocking the prothrombotic effect of erythrocytes with appropriate doses of aspirin.

Experimental data indicate that the prothrombotic effect of erythrocytes is inhibited, in a dose-dependent manner, by 50–500 mg of aspirin; nearly-complete inhibition occurs after two hours of a single dose of 500 mg[14]. Moreover, the antithrombotic effect of 500 mg of aspirin on erythrocytes is reversible after some time and disappears within few days[14]. Therefore, we assessed whether a low daily dose of aspirin could maintain the effect of the initial dose of 500 mg for a longer time. To evaluate this possibility, normal donors were given an initial dose of 500 mg of aspirin, followed by 50 mg/day. This dosage reduced the prothrombotic effect of erythrocytes for 2–3 weeks, but normal conditions were restored after 3–5 weeks[14]. Thus, to control simultaneously the prothrombotic activity of platelets and erythrocytes in normal subjects, a dose of 500 mg

every 15 days along with a daily dose of 50 mg seems to be appropriate[14].

We also assessed to what extent the usual doses of aspirin given in our medio to patients with cardiovascular or cerebrovascular disease (200–300 mg/day) were appropriate to inhibit simultaneously the prothrombotic actions of platelets and erythrocytes. Eighty patients (62 with cardiovascular pathology and 20 with stroke) were assessed. The results showed that these doses of aspirin inhibited TXA_2 synthesis in more than 94% of patients[81]. Nevertheless, when platelet recruitment was assessed, we found that 39% of patients showed complete inhibition, both in the platelets alone and in presence of erythrocytes. In 45% of patients, the response of isolated platelets was completely inhibited, but the inhibition was not enough in the presence of erythrocytes. Finally, suboptimal inhibition of platelet activity was observed in the remaining 16% of patients, in the platelets alone and in the presence of erythrocytes. Therefore, we conclude that, in almost 60% of the patients treated with 200–300 mg aspirin/day, aspirin was not sufficient to block the platelet reactivity in the presence of erythrocytes, despite the inhibition of TXA_2 synthesis. Another finding of interest is that, in aspirin treatment, a larger proportion of cerebrovascular compared with cardiovascular patients show suboptimal control of platelet function when whole blood is assessed. This may indicate that aspirin exerts a different effect on the platelet response in different vascular areas, which could be stronger in coronary than brain areas[99]. Moreover, we have observed that the effect of aspirin is not homogeneous in all patients, consistent with other reports; drug resistance has been reported in some patients[100,101].

In further studies, we proved that 500 mg of aspirin can inhibit the prothrombotic effect of erythrocytes in different groups of patients, as has been observed in normal subjects[14]. In contrast the daily dose of 50 mg/day is clearly not enough in vascular patients, especially in the presence of erythrocytes, although TXA_2 synthesis is inhibited[14,102]. Thus, at present we are assessing the efficacy of a dose of 500 mg every 15 days, and higher daily doses of 100 and 200 mg given once daily, or the same dose divided in two intakes every 12 hours. Preliminary results of this on-going study suggest that better control of platelet recruitment in whole blood is achieved with a loading dose of 500 mg per day every 15 days, with a daily maintaining dose of 100 or 200 mg/day, compared with a dose of 200–300 mg/day given in a continuous manner[103]. These data show that the dose of aspirin is important for the control of the prothrombotic effect of erythrocytes. Furthermore, we have found that the same dose of aspirin given every 12 hours significantly increases the inhibition of platelet recruitment in whole blood. We believe that this could be related to platelet turnover. The daily platelet turnover in normal subjects is about 10%, and turnover increases in patients with atherosclerosis[104-106]. Four hours after aspirin administration, platelets with active cyclooxygenase can be found in the blood circulation[107]. From a functional point of view, it is known that platelet activation can be maintained by a residual capacity of TXA_2 synthesis of 10% in aspirin-trealed platelets; among platelets treated with aspirin only, a small number of functional platelets exhibit increased reactivity, both in vitro and in vivo, in thrombus formation[108,109]. We have also shown that, when only 7% of the platelets are left untreated with aspirin, platelet recruitment occurs. Thus, the effects mentioned above account for better control by aspirin given every 12 hours, since it reduces the number of platelets not treated with aspirin.

At present, it is known that aspirin is effective in a wide range of doses from 50 to 1500 mg/day; in high-risk patients, any of these doses reduces vascular events to a similar extent[95,99]. This is consistent with the hypothesis that the effect of aspirin on platelet COX-1 is saturable and shows no dose-dependence[99]. Nevertheless, the optimal dose of aspirin is still a controversial issue. Since there are gastrointestinal side effects (which are dose-dependent) and risk of hemorrhage (not dose-dependent), it seems advisable to recommend the minimal effective dose in each type of vascular pathology[99,110,111]. There are other effects of aspirin which are not associated with TXA_2 synthesis, such as effects on coagulation, fibrinolysis, trombin generation, or the prothrombotic effect of erythrocytes mentioned above[99]. These effects of aspirin are not related to the synthesis of eicosanoids and are less characterized than the effects of aspirin on COX-1 but probable play also a role on thrombogenesis. The average doses recommended by different scientific consensus inhibit COX-1 in platelets. However as suggested by our data, some of aspirin's mechanisms of action, such as the effects on erythrocytes or leukocytes, may play also an

important role in the occurrence of ischemic and thrombotic events. Thus, to allow prescription of the minimal effective dose, we advise that the antithrombotic effect of aspirin should be assessed in each patient in platelets and in whole blood.

REFERENCES

1. Marcus AJ. Platelets: Their role in hemostasis, thrombosis and inflammation. In: Gallin JI, Snyderman R (eds). Inflammation: Basic principles and Clinical Correlates. Philadelphia: Lippincott Williams & Wilkins, 1999:77-95.
2. Santos MT, Moscardó A, Vallés J, Martínez M, Pinon M, Aznar J, et al. Participation of tyrosine phosphorylation in cytoskeletal reorganization, aIIbß$_{III}$ integrin receptor activation, and aspirin-insensitive mechanisms of thrombin-stimulated human platelets. Circulation 2000;102:1924-30.
3. Santos MT, Vallés J, Aznar J, Perez-Requejo JL. Role of red blood cells in the early stage of platelet activation by collagen. Thromb Haemostas 1986;56: 376-81.
4. Santos MT, Vallés J, Marcus AJ, Safier LB, Broekman MJ, Islam N, et al. Enhancement of platelet reactivity and modulation of eicosanoid production by intact erythrocytes. J Clin Invest 1991;87:571-80.
5. Vallés J, Santos MT, Aznar J, Marcus AJ, Martínez-Sales V, Portoles M, et al. Erythrocytes metabolically enhance collagen-induced platelet responsiveness via increased thromboxane production, ADP release, and recruitment. Blood 1991;78:154-62.
6. Vallés J, Santos MT, Marcus AJ, Safier LB, Broekman MJ, Islam N, et al. Downregulation of human platelet reactivity by neutrophils. Participation of lipoxygenase derivatives and adhesive proteins. J Clin Invest 1993;92:1357-65.
7. Marcus AJ. Thrombosis and inflammation as multicellular processes: Patophysiologic significance of transcellular metabolism. Blood 1990;76:1903-7.
8. Gresele P, Zoja C, Deckmyn H, Arnout J, Vermylen J, Verstraete M. Dipyridamole inhibits platelet aggregation in whole blood. Throm Haemostas 1983;50: 852-6.
9. Pérez-Requejo JL, Aznar J, Santos MT, Vallés J. Early platelet-collagen interactions in whole blood and their modifications by aspirin and dipyridamole evaluated by a new method (Basic Wave). Thromb Haemostas 1985;54:799-803.
10. Rocca B, Fitz Gerald GA. Simply read: Erythrocytes modulate platelet function. Should we rethink the way we give aspirin? Circulation 1997;95:11-3.
11. Bozzo J, Hernandez MR, Alemany M, Rosell G, Bastida E, Escolar G, et al. Effects of aspirin and indomethacin separately in red blood cells and platelets. Modulation of the adhesive and cohesive functions of platelets under flow conditions. Platelets 1996;7: 277-82.
12. López-Farre A, Caramelo C, Esteban A, Alberola ML, Millas I, Monton M, et al. Effects of aspirin on platelet-neutrophil interactions: Role of nitric oxide and endothelin-1. Circulation 1995;91:2080-8.
13. López-Farre A, Riesco A, Digiuni E, Mosquera JR, Caramelo C, Demiguel LS, et al. Aspirin-stimulated nitric oxide production by neutrophils after acute myocardial ischemia in rabbits. Circulation 1996;94:83-7.
14. Santos MT, Vallés J, Aznar J, Marcus AJ, Broekman MJ, Safier LB. Prothrombotic effects of erythrocytes on platelet reactivity: Reduction by aspirin. Circulation 1997;95:63-8.
15. Marcus AJ. Eicosanoid interactions between platelets, endothelial cells and neutrophils. Meth Enzym 1990;187:585-99.
16. Maclouf J, Fitzpatrick FA, Murphy RC. Transcellular biosynthesis of eicosanoids. Pharmacol Res 1989;21:1-7.
17. Marcus AJ, Hajjar DP. Vascular transcellular signaling. J Lipid Res 1993;34:2017-31.
18. Marcus AJ, Weksler BB, Jaffe EA, Broekman MJ. Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. J Clin Invest 1980;66:979-86.
19. Karim S, Habib A, Levy-Toledano S, Maclouf J. Cyclooxygenases-1 and -2 of endothelial cells utilize exogenous or endogenous arachidonic acid for transcellular production of thromboxane. J Biol Chem 1996;271:12042-8.
20. Marcus AJ, Safier LB, Ullman HL, Islam N, Broekman MJ, Falck JR, et al. Platelet-neutrophil interactions (12S)-hydroxyeicosatetraen-1, 20-dioic acid: A new eicosanoid synthesized by unstimulated neutrophils from (12S)-20-dihydroxyeicosatetraenoic acid. J Biol Chem 1988;263:2223-9.
21. Fitzpatrick F, Liggett W, McGee J, Bunting S, Morton D, Samuelsson B. Metabolism of leukotriene A4 by human erythrocytes. A novel cellular source of leukotriene B4. J Biol Chem 1984;259:11403-7.
22. Maclouf J, Murphy RC, Henson PM. Transcellular sulfidopeptide leukotriene biosynthetic capacity of vascular cells. Blood 1989;74:703-7.
23. McGee JE, Fitzpatrick FA. Erythrocyte-neutrophil interactions: Formation of leukotriene B_4 by transcellular biosynthesis. Proc Natl Acad Sci USA 1986; 83:1349-53.
24. Bednar M, Smith B, Pinto A, Mullane KM. Neutrophil depletion suppresses (1) in-labeled platelet accumulation in infarcted myocardium. J Cardiovasc Pharmacol 1985;7:906-12.
25. Geng JG, Bevilacqua MP, Moore KL, Mc Intyre TM, Prescott SM, Kim JM, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature 1990;343:757-60.
26. Guyer DA, Moore KL, Lynam EB, Schammel CMG, Rogelj S, Mc Ever RP, et al. P-selectin glycoprotein li-

gand-1 (PSGL-1) is a ligand for l-selectin in neutrophil aggregation. Blood 1996;88:2415-21.

27. Ward PA, Varani J. Mechanisms of neutrophil-mediated killing of endothelial cells. J Leukocyte Biol 1990;48:97-102.
28. Williams FM. Neutrophils and myocardial reperfusion injury. Pharmacol Ther 1996;72:1-12.
29. Lefer AM, Campbell B, Scalia R, Lefer DJ. Synergism between platelets and neutrophils in provoking cardiac dysfunction after ischemia and reperfusion: Role of selectins. Circulation 1998;98:1322-8.
30. Gillis S, Furie BC, Furie B. Interactions of neutrophils and coagulation proteins. Semin Hematol 1997;34:336-42.
31. Vallés J, Santos MT. Interrelación plaqueta-leucocito. Posibles implicaciones en la patogénesis de los fenómenos trombóticos. In: Lopez-Borrasca A, Arocha-Piñango CL, Campos-Guerra CC, Parreira A, Pavlovski S, Ruiz-Argüelles G, et al (eds). Enciclopedia Iberoamericana de Hematologia. 1st ed. V. III. Salamanca: Ediciones Universidad de Salamanca, 1992.
32. Selak MA, Chignard M, Smith JB. Cathepsin G is a strong platelet agonist released by neutrophils. Biochem J 1988;251:293-9.
33. De Gaetano G, Cerletti C, Evangelista V. Recent advances in platelet-polymorphonuclear leukocyte interaction. Haemostasis 1999;29:41-9.
34. Nicolini FA, Mehta JL. Inhibitory effect of unstimulated neutrophils on platelet aggregation by release of a factor similar to endothelium-derived relaxing factor (EDRF). Biochem Pharmacol 1990;40:2265-9.
35. Salvemini D, de-Nucci G, Gryglewski RJ, Vane JR. Human neutrophils and mononuclear cells inhibit platelet aggregation by releasing a nitric oxide-like factor. Proc Natl Acad Sci USA 1989;86:6328-32.
36. Mc Call TB, Boughton-Smith NK, Palmer RMJ, Whittle BJR, Moncada S. Synthesis of nitric oxide from L-arginime by neutrophils. Release and interaction with superoxide anion. Biochem J 1989;261: 293-6.
37. De La Cruz JP, Blanco E, De la Cuesta FS. Effect of dipyridamole and aspirin on the platelet-neutrophil interaction via the nitric oxide pathway. Eur J Pharmacol 2000;397:35-41.
38. Schattner MA, Geffner JR, Isturiz MA, Lazzari MA. Inhibition of human platelet activation by polymorphonuclear leukocytes. Br J Pharmacol 1990;101: 253-6.
39. Oudinet JP, Sraer J, Bens M, Ardaillou R. Influence of polymorphonuclear leukocytes on the metabolism of arachidonate in human platelets. Thromb Haemostas 1988;60:59-62.
40. Levine PH, Weinger RS, Simon J, Scoon KL, Krinsky NI. Leukocyte-platelet interaction. Release of hydrogen peroxide by granulocytes as a modulator of platelet reactions. J Clin Invest 1976;57:955-63.
41. Stuart MJ, Holmsen H. Hydrogen peroxide, an inhibitor of platelet function: Effect on adenine nucleotide metabolism, and the release reaction. Am J Hematol 1977;2:53-63.
42. Marcus AJ. Neutrophils inhibit platelet reactivity by multiple mechanisms. Relevance to thromboregulation. J Lab Clin Med 1990;116:138-9.
43. McEver RP. Selectins: Novel receptors that mediate leukocyte adhesion during inflammation. Thromb Haemostas 1991;65:223-8.
44. Yang J, Furie BC, Furie B. The biology of P-selectin glycoprotein ligand-1: Its role as a selectin counterreceptor in leukocyte-endothelial and leukocyte-platelet interaction. Thromb Haemost 1999;81:1-7.
45. Marcus AJ, Safier LB. Thromboregulation- multicellular modulation of platelet reactivity in hemostasis and thrombosis. FASEB J 1993;7:516-22.
46. Michelson AD, Barnard MR, Khuri SF, Rohrer MJ, Macgregor H, Valeri CR. The effects of aspirin and hypothermia on platelet function in vivo. Brit J Haematol 1999;104:64-8.
47. Chronos NAF, Wilson DJ, Janes SL, Hutton RA, Buller NP, Goodall AH. Aspirin does not affect the fow cytometric detection of fibrinogen binding to, or release of alpha-granules or lysosomes from, human platelets. Clin Sci 1994;87:575-80.
48. Palabrica T, Lobb R, Furie BC, Aronowitz M, Benjamin C, Hsu YM, et al. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. Nature 1992;359:848-51.
49. Santos MT, Aznar J. Interacción plaquetas-hematíes en la patogenia de la trombosis. In: Lopez-Borrasca A, Arocha-Piñango CL, Campos-Guerra CC, Parreira A, Pavlovski S, Ruiz-Argüelles G, et al, (eds). Enciclopedia Iberoamericana de Hematologia. 1st ed. v. III. Salamanca: Ediciones Universidad de Salamanca, 1992.
50. Duke WW. The relation of blood platelets to hemorrhagic disease. JAMA 1910;60:1183-92.
51. Lowe GDO, Forbes CD. Platelet aggregation, haematocrit, and fibrinogen. Lancet 1985;1:395-6.
52. Editorial. The Bleeding-time and haematocrit (Editorial). Lancet 1984;1:997-8.
53. Moia M, Vizzotto L, Cattaeno M, Mannucci PM, Casati S, Ponticelli C. Improvement in the haemostatic defect of uraemia after treatment whith recombinant human erythropoietin. Lancet 1987;28:1227-9.
54. Crowley JP, Metzger JB, Valeri CR. The volume of blood shed during the bleeding time correlates with the peripheral venous hematocrit. Am J Clin Pathol 1997;108:579-84.
55. Thomas DJ, Marshall J, Ross Russell RW. Effects of hematocrit on cerebral blood flow in man. Lancet 1977;2:941-3.
56. Pearson TC, Wetherley-Mein G. Vascular occlusive episodes and venous haematocrit in primary proligerative polycythaemia. Lancet 1978;2:1219-22.
57. Tohgi H, Yamanousi H, Murakami M, Kameyama M.

Importance of the hematocrit as a risk factor in cerebral infarction. Stroke 1978;9:369-74.

58. Boneu B. Laboratory investigations and prediction of thrombotic risk in polycythemia vera. Nouv Rev Fr Hematol 1994;36:183-5.
59. Spiess BD, Ley C, Body SC, Siegel LC, Stover EP, Maddi R, et al. Hematocrit value on intensive care unit entry influences the frequency of q-wave myocardial infarction after coronary artery bypass grafting. J Thorac Cardiovasc Surg 1998;116:460-7.
60. Carter C, McGee D, Reed D, Yano K, Stemmermann G. Hematocrit and the risk of coronary heart disease: The Honolulu heart program. American Heart Journal 1983;105:674-9.
61. Cirillo M, Capasso G, Desanto NG. Relationship between hematocrit and blood pressure implications for primary hypertension. Nephron 1993;65:505-10.
62. Vallés J, Santos MT, Aznar J, Beltrán M, Beltrán C, Martínez-Sales V, et al. Platelet-erythrocyte interactions in hypercholesterolemic patients. Haemostasis 1996;26(Suppl 3):461.
63. Vallés J, Santos MT, Aznar J, Velert M, Barbera G, Carmena R. Modulatory effect of erythrocytes on the platelet reactivity to collagen in IDDM patients. Diabetes 1997;46:1047-53.
64. Koenig W, Ernst E. The possible role of hemorheology in atherothrombogenesis. Atherosclerosis 1992;94:93-107.
65. Turitto VT, Weiss HJ. Red blood cells: Their dual role in thrombus formation. Science 1980;207:541-3.
66. Weiss HJ. Flow-related platelet deposition on subendothelium. 1995;74:117-22.
67. Aarts PA, Bolhuis PA, Sakariassen KS, Heethaar RM, Sixma JJ. Red blood cell size is important for adherence of blood platelets to artery subendothelium. Blood 1983;62:214-7.
68. Willems C, Stel HV, van-Aken WG, van-Mourik JA. Binding and inactivation of prostacyclin (PGI-2) by human erythrocytes. Br J Haematol 1983;54:43-52.
69. Jakubowski JA, Thompsom CB, Deykin D. Inactivation of prostacyclin (PGI-2) by erythrocytes. Br J Haematol 1983;54:658-60.
70. Houston DS, Robinson P, Gerrard JM. Inhibition of intravascular platelet aggregation by endothelium-derived relaxing factor: Reversal by red blood cells. Blood 1990;76:953-8.
71. Evans HG, Ryley HC, Hallet I, Lewis MJ. Human red blood cells inhibit endotheliun-derived relaxing factor (EDRF) activity. Eur J Pharmacol 1989;163: 361-4.
72. Marcus AJ, Broekman MJ, Drosopoulos JHF, Islam N, Alyonycheva TN, Safier LB, et al. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. J Clin Invest 1997;99:1351-60.
73. Reimers RC, Sutera SP, Joist JH. Potentiation by red blood cells of shear-induced platelet aggregation: Relative importance of chemical and physical mechanisms. Blood 1984;64:1200-6.
74. Goldsmith HL, Bell DN, Braovac S, Steinberg A, Mc Intosh F. Physical and chemical effects of red cells in the shear-induced aggregation of human platelets. Biophys J 1995;69:1584-95.
75. Gaarder A, Jonsen J, Laland S, Hellem A, Owren PA. Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelelets. Nature 1961;192:531-2.
76. Hellem AJ, Borchgrevink CF, Ames SH. The role of red cells in haemostasis: The relation between haematocrit, bleeding time and platelet adhesiveness. Br J Haematol 1961;7:42-50.
77. Born GV, Wehmeier A. Inhibition of platelet thrombus formation by chloropromacine acting to diminish haemolysis. Nature 1979;282:212-3.
78. Weiss HJ, Lages B, Hoffmann T, Turitto VT. Correction of the platelet adhesion defect in delta-storage pool deficiency at elevated hematocrit-possible role of adenosine diphosphate. Blood 1996;87:4214-22.
79. Saniabadi AR, Lowe GD, Barbenel JC, Forbes CD. A comparison of spontaneous platelet aggregation in whole blood with platelet rich plasma: Additional evidence for the role of ADP. Thromb Haemost 1984;51:115-8.
80. Setiabudy-Dharma R, Funahara Y. Enhancement of collagen-induced aggregation of platelets in whole blood. 1986;42:621-34.
81. Vallés J, Santos MT, Aznar J, Osa A, Lago A, Cosín J, et al. Erythrocyte promotion of platelet reactivity decreases the effectiveness of aspirin as an antithrombotic therapeutic modality: The effect of low-dose aspirin is less than optimal in patients with vascular disease due to prothrombotic effects of erythrocytes on platelet reactivity. Circulation 1998;97:350-5.
82. Escolar G, Cases A, Vinas M, Pino M, Calls J, Cirera I, et al. Evaluation of acquired platelet dysfunctions in uremic and cirrhotic patients using the platelet function analyzer [PFA 100 (TM)]: Influence of hematocrit elevation. Haematologica 1999;84:614-9.
83. Marcus AJ, Safier LB, Ullman HL, Islam N, Broekman MJ, Falck JR, et al. Interactions between platelets and neutrophils in the eicosanoid pathway. In: Samuelsson B, Wong PYK, Sun FF (eds). Advances in Prostaglandin, Thromboxane, and Leukotriene Research. V. 19. New York: Raven Press Ltd, 1989:263-6.
84. Buchanan MR, Horsewood P, Brister SJ. Regulation of endothelial cell and platelet receptor-ligand binding by the 12- and 15-lipoxygenase monohydroxides, 12-, 15-HETE and 13-HODE. Prostagland Leuk Essent Fatty 1998;58:339-46.
85. Honn KV, Grossi IM, Diglio CA, Wojtukiewicz M, Taylor JD. Enhanced tumor cell adhesion to the subendothelial matrix resulting from 12(S)-HETE-induced endothelial cell retraction. FASEB J 1989;3:2285-93.
86. Iuliano L, Violi F, Pedersen JZ, Pratico D, Rotilio G, Balsano F. Free radical-mediated platelet activation by he-

moglonin released from red blood cells. Archives of Biochemistry and Biophysics 1992;299:220-4.

87. Violi F, Iuliano L, Balsano F. Iron, platelet function, and coronary heart disease - A possible link. Circulation 1993;88:805-6.
88. Vallés J, Santos MT, Martínez M, Moscardó A, Gómez-Biedma S, Piñón M, et al. Erythrocytes increase GP IIb IIIa receptor activation and secretion in recruiting platelets. Haemostasis 2000;30(Suppl 1):78A.
89. Moscardó A, Vallés J, Santos MT, Piñón M, Aznar J. Papel regulador de la interacción eritrocito-plaqueta en los mecanismos intracelulares de transmisión de señales. Haematologica 2000;85(Suppl 3):19A.
90. Li Q, Jungmann V, Kiyatkin A, Low PS. Prostaglandin E_2 stimulates a ca2+-dependent k+ channel in human erythrocytes and alters cell volume and filterability. J Biol Chem 1996;271:18651-6.
91. Gilboagarber N, Kirsteinsegal R. Structural specificity of serotonin effect on human erythrocyte fragility. Mol Genet Metab 1998;64:283-5.
92. Jain SK, Ross JD, Levy GJ, Duett J. The effect of malonyldialdehyde on viscosity of normal and sickle red blood cells. Biochem Med Metab Biol 1990;44:37-41.
93. Bozzo J, Hernandez MR, Ordinas A. Reduced red cell deformability associated with blood flow and platelet activation: Improved by dipyridamole alone or combined with aspirin. Cardiovasc Res 1995;30: 725-30.
94. Fuster V. Mechanisms of arterial thrombosis: Foundation for therapy. Am Heart J 1998;135(Suppl 6): 361-6.
95. Antiplatelet Trialists' Collaboration. Collaborative overview of randomized trials of antiplatelet therapy- I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Br Med J 1994;308:81-106.
96. Patrono C, Ciabattoni G, Patrignani P, Pugliese F, Filabozzi P, Catella F, et al. Clinical pharmacology of platelet cyclooxygenase inhibition. Circulation 1985;72:1177-84.
97. Marcus AJ. Platelets and their disorders. In: Ratnoff OD, Forbes CD (eds). Disorders of Hemostasis. 3rd ed. Philadelphia: WB Saunders, 1996:79-137.
98. Coller BS. Platelets in cardiovascular thrombosis and thrombolysis. In: Frozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE (eds). The Heart and Cardiovascular System. 2nd ed. New York: Raven Press, 1992:219-73.
99. Patrono C, Coller B, Dalen JE, Fitz Gerald GA, Fuster V, Gent M, et al. Platelet-active drugs: The relationships among dose, effectiveness, and side effects. Chest 2001;119(Suppl 1):39-63.
100. Helgason CM, Tortorice KL, Winkler SR, Penney DW, Schuler JJ, McClelland TJ, et al. Aspirin response and failure in cerebral infarction. Stroke 1993;24:345-50.
101. Helgason CM, Bolin KM, Hoff JA, Winkler SR, Mangat A, Tortorice KL, et al. Development of aspirin resistance in persons with previous ischemic stroke. Stroke 1994;25:2331-6.
102. Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest 1982;69:1366-72.
103. Santos MT, Vallés J, Aznar J, Lago A, Martínez-Sales V, Moscardó A, et al. Aspirin treatment in patients with vascular diseases: A study aimed at a better control of platelet reactivity. Haemostasis 2000;30(Suppl 1):86A.
104. Harker LH, Finch CA. Thrombokinetics in man. J Clin Invest 1969;48:963-74.
105. Minar E, Ehringer H, Ahmadi R, Dudczak R, Porenta G. Platelet deposition at angioplasty sites and platelet survival time after PTA in iliac and femoral arteries: Investigations with indium-111-oxine labelled platelets in patients with ASA (1.0 g/day)-therapy. Thromb Haemost 1987;58:718-23.
106. Wessels P, Heyns AD, Esterhuysen AJ, Badenhorst PN, Lotter MG, Pieters H, et al. Kinetics and in vivo distribution of in-111-labelled platelets and platelet function in familial hypercholesterolaemia. Thromb Haemost 1987;58:811-6.
107. Di Minno G, Silver MJ, Murphy S. Monitoring the entry of new platelets into the circulation after ingestion of aspirin. Blood 1983;61:1081-5.
108. Reilly IA, Fitz Gerald GA. Inhibition of thromboxane formation in vivo and ex vivo: Implications for therapy with platelet inhibitory drugs. Blood 1987;69:180-6.
109. Cerskus AL, Ali M, Davies BJ, Mc Donald JV. Possible significance of small numbers of functional platelets in a population of aspirin-treated platelets in vitro and in vivo. Thromb Res Suppl 1980;18:389-97.
110. Cairns JA, Theroux P, Lewis HD, Ezekowitz M, Meade TW. Antithrombotic agents in coronary artery disease. Chest 2001;119(Suppl 1):228-52.
111. Albers GW, Amarenco P, Easton JD, Sacco RL, Teal P. Antithrombotic and thrombolytic therapy for ischemic stroke. Chest 2001;119(Suppl 1):300-20.

Address for Correspondence:

Justo AZNAR, MD

Departamento de Biopatologia Clínica
Hospital Universitario La Fe
Avda. Campanar 21
46009, Valencia, SPAIN