Biochemical Pharmacology, Vol. 22, pp. 3151–3156. Pergamon Press, 1973. Printed in Great Britain.

COMMENTARY

DRUGS INHIBITING PLATELET FUNCTION

J. FRASER MUSTARD and MARIAN A. PACKHAM

Department of Pathology, McMaster University, Hamilton, Ontario and Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada

PLATELETS are involved in the intravascular responses to vessel injury, foreign surfaces and particles, viruses, bacteria, and antigen–antibody complexes.[1] The most generally recognized process in which platelets are considered to play an important part is the development of arterial thrombi. There is, however, evidence that platelets may be involved in other processes as well, such as vessel injury during serum sickness, and reactions that occur in transplanted kidneys.[1] The principal reactions of platelets are:

(1) interaction with subendothelial structures such as collagen, microfibrils and the basement membrane;
(2) release of granule constituents when stimulated with collagen, thrombin, antigen–antibody complexes, foreign particles in the blood, etc.;
(3) alteration in shape in response to ADP, preceding aggregation;
(4) acceleration of blood coagulation following aggregation by ADP or collagen;
(5) adherence to polymerizing fibrin.

There are a number of compounds which inhibit these reactions, but only a few of these are suitable for administration to man in effective doses.

Interaction of platelets with surface stimuli

The non-steroidal anti-inflammatory and related drugs[1–3] inhibit the interaction of platelets with collagen, antigen–antibody complexes, viruses, bacteria, gamma globulin-coated surfaces, fibrinogen-coated surfaces and dialysis membranes.[4] At concentrations which inhibit the interaction of platelets with these agents, these drugs do not inhibit primary ADP-induced aggregation, nor are they very effective against the release reaction caused by thrombin.

Studies with some of the compounds such as aspirin have shown that these drugs do not increase the cyclic AMP concentration in platelets, nor do they exert their effect by inhibiting metabolism. They do inhibit collagen glucosyl transferase on the surface of platelets,[5] and they inhibit the synthesis of prostaglandins E_2 and $F_{2\alpha}$[6] when platelets are stimulated with release-inducing stimuli. It seems unlikely, however, that their inhibitory effect is exerted through their inhibition of the synthesis of these prostaglandins because aspirin, at a concentration that inhibits thrombin-induced prostaglandin synthesis, does not block the release reaction or platelet aggregation.[6]

Aspirin differs from the other drugs in that it has been shown to acetylate platelet proteins and the effect of aspirin cannot be removed by washing the platelets. Aspirin has long-lasting effects on platelet function following oral administration and this is most probably related to its ability to acetylate platelet membrane components. In the case of all the other drugs in this group, the effects on platelet function disappear in parallel with the loss of the compound from the plasma.

There is evidence that aspirin, sulfinpyrazone, or phenylbutazone inhibits the adhesion of platelets to fibrinogen-coated or collagen-coated surfaces.[2,7] It may be that their primary mode of action is inhibition of the interaction of platelets with particulate matter or surfaces; this may account for the relatively weak effect of these drugs on thrombin-induced release and aggregation.

There seems little doubt that aspirin prolongs the bleeding time in man and that it and similar drugs aggravate hemorrhagic tendencies in subjects with hemophilia.[1–3] A correlation has been shown between the duration of aspirin's inhibition of collagen-induced aggregation as tested *in vitro* and the length of time during which increased bleeding times can be observed in man.[8] In animal experiments, although most investigators observed prolongation of bleeding time by aspirin and other anti-inflammatory drugs, Arfors *et al.*[9] have published negative results. Animal experiments designed to study the effect of these drugs in a variety of situations have indicated that with renal transplants, arterial injury, or extracorporeal shunts, the drugs inhibit the extent of thrombus formation.[1,2] In considering the negative results, it is important to bear in mind that the flow conditions may have a major effect on thrombus formation and differ a great deal in studies by various investigators. Mechanical or chemical injury of vessels may have a different effect than injury with an electrical current or a laser beam. The latter stimuli may damage the red cells and cause loss of ADP from them. It is important to recognize that the anti-inflammatory drugs do not inhibit primary ADP-induced aggregation and therefore they would not be expected to have much effect in such circumstances.

There is widespread interest in the use of these drugs for the management of thromboembolic complications in man, particularly since the drugs are readily available, inexpensive and have a relatively low toxicity. The effect of aspirin on venous thrombosis is controversial.[2] Recently, Genton *et al.*[10] have found that sulfinpyrazone will prolong shortened platelet survival times toward normal values and will reduce the incidence of episodes of thrombosis in individuals with recurrent venous thrombosis. In some patients with certain types of prosthetic heart valves, platelet survival times are shortened, and this is associated with an increase in the number of thromboembolic episodes. Aspirin by itself has not been found to restore platelet survival to normal, whereas sulfinpyrazone has.[2] It is difficult to understand why sulfinpyrazone appears to be effective in venous thrombosis in patients with prosthetic heart valves while aspirin is not. If this difference is substantiated in further studies, it would indicate that there must be a fundamental difference in the mode of action of these two drugs.

The few brief studies of the effect of these drugs on the management of conditions in which arterial thrombosis or thromboembolism is believed to be important, such as transient attacks of cerebral ischemia and amaurosis fugax, have indicated that both aspirin and sulfinpyrazone are effective.[11,12] Sulfinpyrazone has been found to reduce significantly the mortality from vascular disease in a group of elderly patients in a

chronic care unit in comparison to a group given a placebo compound.[13] Furthermore, Pineo *et al.*[14] found that sulfinpyrazone treatment of patients on hemodialysis therapy significantly reduced the amount of thrombus formation in forearm shunts compared with the group given a placebo. It appears that, in situations where fairly direct evidence is available about the thrombotic process, both aspirin and sulfinpyrazone are effective.

It should be kept in mind that the influence of these compounds on the complications of vascular disease may not be solely mediated by their effects on platelets. It is conceivable, for example, that they may influence the endothelium and protect it from injury, thereby diminishing the frequency of thrombosis.

ADP-induced platelet aggregation

Many of the compounds reported to inhibit ADP-induced aggregation inhibit only the secondary phase.[1–3] It is important to ascertain whether the compounds are inhibiting primary or secondary aggregation when using ADP with human citrated platelet-rich plasma. Failure to do this may lead to misinterpretation of the effects of drugs; some may only be inhibiting the release reaction that is involved in the second phase of ADP-induced aggregation. There are very few drugs that can be used clinically which inhibit the primary phase of ADP-induced aggregation. The most potent agent for inhibiting ADP-induced primary aggregation is prostaglandin E_1.[1–3] Unfortunately, it is rapidly cleared from the circulation and hence has little usefulness for administration *in vivo*. Prostaglandin E_1 also inhibits the release reaction, but this effect may be due to its action on ADP rather than on the release reaction itself. It is known that with low concentrations of release-inducing stimuli, the small amount of ADP which is released has an enhancing effect on further release.[15] Elimination of this ADP effect causes a significant reduction in the extent of release when stimuli are used at low concentrations. Prostaglandin E_1 does not inhibit release when it is caused by high concentrations of release-inducing stimuli. A practical application of this action of prostaglandin E_1 is its use in the preparation of platelet concentrates for transfusions because it prevents aggregate formation during processing.[16] Prostaglandin E_1 maintains platelets in a disc-shape, accelerates deaggregation, and permits platelet recovery even after thrombin-induced release and aggregation.[17] Platelets in concentrates prepared with PGE_1 survive normally after transfusion and the negligible amount of PGE_1 infused disappears rapidly.[16]

The pyrimido–pyrimidine compounds also may exert some of their inhibitory effects by increasing platelet cyclic AMP levels.[2,3] These compounds include dipyridamole, RA 233, RA 433, VK 744, VK 774* and others. In fairly high concentrations, these compounds inhibit the primary phase of ADP-induced platelet aggregation. They inhibit the platelet release reaction, including that induced by thrombin, but this may be related to their effect on primary ADP-induced aggregation. With the exception of RA 433, they have not been found to be very effective inhibitors of platelet adherence to collagen-coated or fibrinogen-coated surfaces.

* RA 233 = 2,6-bis-(diethanolamino)-4-piperidino-pyrimido-[5,4-d]-pyrimidine; RA 433 = 2,4,6-trimorpholinopyrimido-[5,4-*d*]-pyrimidine; VK 744 = 2-[(2-aminoethyl) amino]-4-morpholinothieno (3,2-D) pyrimidine-dihydrochloride; VK 774 = 4-morpholino-2-piperazino-thieno-(3,2-D) pyrimidine-dihydrochloride.

Drugs in this class have been reported to inhibit thrombus formation in injured vessels of experimental animals according to some investigators but not others.[2] There are a number of problems in the use of these compounds because there are species differences in the effects of the drugs and there is the complicating effect of the drugs on blood pressure. In high doses, these compounds have been shown to inhibit thrombus formation in extracorporeal shunts[2] and to reduce microaggregate formation in blood circulated for a long period in pump oxygenators connected to dogs.[18]

The results of the studies in man are confusing.[1,2] Dipyridamole by itself does not appear to be effective in the management of thromboembolic complications of atherosclerotic vascular disease. It has been reported to be useful in the management of thromboembolism caused by the artificial surfaces of vascular prosthetic devices and to restore platelet survival times to normal in subjects with a history of embolism. In these studies, many of the patients were receiving anticoagulants as well as dipyridamole. Kincaid-Smith[19] has reported that dipyridamole combined with heparin or phenindione prevented thrombosis and vessel narrowing in patients with cadaveric renal allografts. Aspirin and dipyridamole together have been found to influence platelet survival in subjects with prosthetic heart valves[20] and the combination has been reported to be successful in the treatment of a patient with thrombotic microangiopathy.[21] Although there may be some reservations about the value of this drug in the management of thrombosis when used by itself, the evidence does indicate that it is effective when it is used in combination with an anticoagulant or possibly with another drug such as aspirin. This raises the whole question of the use of combinations of drugs which alter different platelet functions, in the attempts to control thrombosis.

Penicillin G

High concentrations of penicillin G inhibit platelet aggregation and release caused by ADP, collagen or thrombin in platelet-rich plasma.[22] This antibiotic also inhibits platelet adhesion to collagen-coated surfaces. An increased bleeding tendency has been reported in some patients who were receiving massive doses of penicillin or related antibiotics. It is possible that some of the effects of massive doses of penicillin in the treatment of bacterial endocarditis may be mediated through its inhibition of platelet reactions involved in the formation of the microthrombi associated with the valvular lesions.

Dextran

There is good evidence[1,23] that 4–6 hr after dextran administration to humans, platelet adhesiveness is reduced; this corresponds to the time when prolongation of the bleeding time can be demonstrated. Despite this, the addition of low molecular weight dextran (40,000–70,000) to platelet-rich plasma *in vitro* does not lead to any change in platelet adhesion, aggregation or release. However, although there are some contradictory reports, in the majority of studies in which dextran has been given during or immediately after surgery, the incidence of thromboembolic complications has been reduced. It is not clear whether dextran acts through its effect on platelets or on some other as yet unidentified mechanism.

Other drugs

There are a great many other drugs which affect platelet function.[1–3] In most cases,

the amounts required to inhibit platelet reactions are higher than can be tolerated *in vivo*. However, some can be used in concentrations which affect platelet functions and some have been found to influence either experimental thrombosis in animals or arterial thrombotic complications in man. Of these, hydroxychloroquine[24] has been reported to reduce the incidence of deep venous thrombosis and pulmonary embolism post-operatively; cyproheptadine[25,26] inhibited thrombosis of injured vessels in the hamster cheek pouch and the accumulation of platelets in renal transplants; nitrofurantoin (Furadantin)[27] or glyceryl guaiacolate[28] can be given to man in doses that inhibit ADP-induced aggregation; pyridinolcarbamate[29] inhibits the enhanced response of platelets to ADP following stress or other conditions in animals and patients. Clofibrate (Atromid S)[1,2] reduces platelet adhesiveness and prolongs platelet survival in man and has been recently found to reduce morbidity and mortality in subjects with ischemic heart disease.[30] Since this effect on ischemic heart disease was not directly related to the changes in circulating lipids, it is conceivable that the effect may be attributable to the rather mild influence this compound has on platelet function.

Other compounds[1–3] which have been shown to affect platelets are: the tricyclic anti-psychotic and anti-depressant drugs (e.g. chlorpromazine, imipramine and amitriptyline), volatile general anesthetics and local anesthetics. The effect of the general anesthetics could be of some significance, particularly if other anti-thrombotic compounds are used as well; under certain circumstances, these anesthetics contribute to bleeding during operations.

With such a long list of compounds that influence platelet function, it is apparent that the effects of combinations of drugs on platelets may be important from the standpoint of the initiation of hemorrhagic complications. Such combinations may also have some practical value in the management of thromboembolic complications. There has been relatively little examination of this problem. However, if one considers the nature of the response of platelets to low concentrations of release-inducing stimuli, some of the potential of this approach becomes apparent. In this situation, the release-inducing stimulus causes release, but if the effect of the released ADP is removed, the extent of release is greatly reduced.[15] The addition of further ADP will enhance the extent of the release reaction. (When high concentrations of a release-inducing stimulus are used, this effect of ADP is not apparent.) Thus, for low concentrations of release-inducing stimuli, one could use a drug which inhibited the action of ADP or one which inhibited the interaction of platelets with the release-inducing stimulus. Both drugs used in combination and at lower concentrations could be effective in inhibiting the release reaction. We have experimental evidence indicating that this can be done. In addition, clinical studies have shown that the use of drugs which influence platelets in combination with anticoagulants can have both harmful and beneficial effects.[1,2] It was recognized earlier that, when drugs such as aspirin were given to subjects receiving oral anticoagulants, hemorrhagic problems frequently occurred. More recently, studies with dipyridamole in patients receiving anticoagulants indicate that this treatment regimen may be effective in handling thromboembolic problems associated with vascular disease and vascular prosthetic devices. Clearly, more investigation is required to analyse this particular approach to handling the potential beneficial and harmful effects of compounds which affect platelets and the blood coagulation mechanism.

REFERENCES

1. J. F. MUSTARD and M. A. PACKHAM, *Pharmac. Rev.* **22,** 97 (1970).
2. J. F. MUSTARD, R. L. KINLOUGH-RATHBONE, C. S. P. JENKINS and M. A. PACKHAM, *Ann. N.Y. Acad. Sci.* **201,** 343 (1972).
3. D. C. B. MILLS, *Clinics in Haematology* (Ed. J. R. O'BRIEN), Vol. 1, p. 295, Saunders, London (1972).
4. R. M. LINDSAY, C. R. M. PRENTICE, D. FERGUSON, J. A. BURTON and G. P. MCNICOL, *Lancet* **2,** 1287 (1972).
5. G. A. JAMIESON, C. L. URBAN and A. J. BARBER, *Nature New Biol.* **234,** 5 (1971).
6. J. B. SMITH and A. L. WILLIS, *Nature New Biol.* **231,** 235 (1971).
7. J.-P. CAZENAVE, M. A. PACKHAM and J. F. MUSTARD, *Third Congress, International Society on Thrombosis and Haemostasis*, Washington, D.C., p. 178 (1972).
8. J. HIRSH, D. STREET, J. F. CADE and H. AMY, *Blood* **41,** 369 (1973).
9. K.-E. ARFORS, D. BERGQVIST, S. BYGDEMAN, F. N. MCKENZIE and E. SVENSJÖ, *Scand. J. Haemat.* **9,** 322 (1972).
10. G. GENTON, H. S. WEILY and P. P. STEELE, *Can. med. Ass. J.* **108,** 463 (1973).
11. M. J. G. HARRISON, J. MARSHALL, J. C. MEADOWS and R. W. ROSS-RUSSELL, *Lancet* **2,** 743 (1971).
12. G. EVANS, *Cerebral Vascular Diseases, Eighth Conference* (Eds. F. H. MCDOWELL and R. W. BRENNAN) p. 297. New York, Grune & Stratton (1973).
13. J. A. BLAKELY, N. SHETH, M. VAVRIK, R. G. B. TAN, H. A. SMYTHE and M. GENT, *Can. med. Ass. J.* **108,** 464 (1973).
14. G. PINEO, A. KAEGI, A. SHIMIZU, H. TRIVEDI and J. HIRSH, *Fourth Congress, International Society on Thrombosis and Haemostasis*, Vienna, p. 168 (1973).
15. M. A. PACKHAM, M. A. GUCCIONE, P.-L. CHANG and J. F. MUSTARD, *Am. J. Physiol.* **225,** 38 (1973).
16. H. SHIO and P. W. RAMWELL, *Science, N.Y.* **175,** 536 (1972).
17. H. J. REIMERS, M. A. PACKHAM, R. L. KINLOUGH-RATHBONE and J. F. MUSTARD, *Br. J. Haemat.*, in press.
18. E. A. RITTENHOUSE, E. A. HESSEL II, C. S. ITO and K. A. MERENDINO, *Ann. Surg.* **175,** 1 (1972).
19. P. KINCAID-SMITH, *Lancet* **2,** 920 (1969).
20. L. A. HARKER and S. J. SLICHTER, *New Engl. J. Med.* **287,** 999 (1972).
21. M. GIROMINI, C. A. BOUVIER, R. DAMI, M. DENIZOT and M. JEANNET, *Br. med. J.* **1,** 545 (1972).
22. J.-P. CAZENAVE, M. A. PACKHAM, M. A. GUCCIONE and J. F. MUSTARD, *Proc. Soc. exp. Biol. Med.* **142,** 159 (1973).
23. H. J. WEISS, *J. Lab. clin. Med.* **69,** 37 (1967).
24. A. E. CARTER, R. EBAN and R. D. PERRETT, *Br. med. J.* **1,** 312 (1971).
25. D. H. MINSKER, P. T. JORDAN and A. MACMILLAN, *Proc. Platelet Symposium, Physiological Society of Philadelphia*, in press.
26. L. RATTAZZI, M. N. HAIMOV, S. GLABMAN, A. PAPATESTAS, I. M. GELERNT and L. BURROWS, *Surg. Forum* **21,** 241 (1970).
27. E. C. ROSSI and N. W. LEVIN, *Third Congress, International Society on Thrombosis and Haemostasis*, Washington, D.C., p. 242 (1972).
28. J. L. SILVERMAN and H. A. WURZEL, *Am. J. clin. Path.* **51,** 35 (1969).
29. H. YAMAZAKI, I. KOBAYASHI and T. SHIMAMOTO, *Thromb. Diath. haemorrh.* **24,** 438 (1970).
30. Five year Study by a Group of Physicians of the Newcastle-upon-Tyne Region, *Br. med. J.* **2,** 767 (1971).