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Perspective

Biological Significance and Therapeutic Potential of Prostacyclin

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During the Autumn of 1975 in collaboration with our colleagues, Richard Gryglewski and Stuart Bunting, we started a line of research which led to the discovery of one of the most exciting substances in the prostaglandin family.[1] The original concept was to determine whether the vessel wall was able to synthesize thromboxane A_2 (the powerful vasoconstrictor and inducer of platelet aggregation generated by platelets), as we believed that the presence of TXA_2 in the vasculature could explain the vasoconstriction which immediately follows cutting of a small vessel. The project showed that thromboxane A_2 was not formed. However, it demonstrated that the prostaglandin endoperoxide PGH_2, the precursor used in the reaction, was consumed and there was no end product detectable by our standard bioassay system, thus suggesting the generation of an unknown biological product.

We spent several unsuccessful weeks trying to pinpoint the nature of this compound which we started to call "PGX". During these weeks we gathered information on the vasodilator effect of PGX since the crude extract was able to relax vascular strips in vitro. A few weeks later we concluded that the vessel wall might by synthesizing the biological counterpart of TXA_2, as a defense mechanism against platelet aggregation, and, as a result of this idea, we tested PGX as an inhibitor of platelet aggregation. It was extremely potent in this respect!

Later work further characterized PGX; it relaxed vascular strips in vitro, caused a vasodilation in vivo, was the most potent inhibitor of platelet aggregation known, and possessed antithrombotic properties. Furthermore, it was the major metabolite of arachidonic acid in vascular tissue. The work which led to the elucidation of the structure of PGX was carried out as a collaborative effort between our own scientists and those from the Upjohn Company at Kalamazoo. PGX was then renamed prostacyclin with the abbreviation of PGI_2.[2-4]

Prostacyclin and thromboxane A_2 are both derived from arachidonic acid, a fatty acid present in the phospholipids of cell membranes. Thromboxane A_2 is an unstable ($t_{1/2}$ = 30 s at 37 °C), powerful vasoconstrictor agent generated by platelets,[5] while prostacyclin is also unstable ($t_{1/2}$ = 3 min at 37 °C) but induces vasodilatation and inhibits platelet aggregation.[1,3] Prostacyclin and thromboxane A_2 represent, therefore, in biological terms, the opposite poles of the same general homeostatic mechanism for regulation of platelet aggregability in vivo. Manipulation of this control mechanism will affect thrombus and hemostatic plug formation[3] (for structures see Figure 1).

The generation of thromboxane A_2 in platelets is inhibited by aspirin and other aspirin-like drugs, and this is why, prior to the discovery of prostacyclin, aspirin was widely promoted as an antithrombotic drug. However, it is now clear that these drugs also inhibit prostacyclin formation in the vessel wall and therefore might have the opposite effect. The utilization of aspirin as a pharmacological tool to investigate the interaction between prostacyclin and thromboxane A_2, however, has been fruitful. Aspirin is highly active against platelet cyclooxygenase in vivo and in vitro. Whereas the analgesic and antiinflammatory dose in humans is about 1.5 g a day, a single tablet of aspirin (325 mg) inhibits the cyclooxygenase of platelets by about 90%. Moreover, this effect is long lasting because aspirin acetylates the active site of the enzyme, causing irreversible inhibition. Platelets are unable to synthesize new protein and cannot replace the cyclo-oxygenase. Therefore, the inhibition will only be overcome by new platelets entering the circulation after the block of cyclo-oxygenase in megakaryocytes has worn off. It is interesting that the cyclo-oxygenase of vessel walls, in vitro and in vivo, seems less sensitive to aspirin than does that of platelets. There is also evidence that vascular tissue in vitro and in vivo recovers from aspirin inhibition by regeneration of its cyclo-oxygenase.

Studies in rabbits suggest that low doses of aspirin reduce TXA_2 formation to a greater extent than prostacyclin formation, thus upsetting the balance and increasing the bleeding time. These experiments also showed that inhibition of TXA_2 formation is longer lasting than that of prostacyclin. In addition, aspirin in high doses (200 mg/kg) increases thrombus formation in a model of venous thrombosis, and in vitro treatment of endothelial cells with aspirin enhances thrombin-induced platelet adherence to them.

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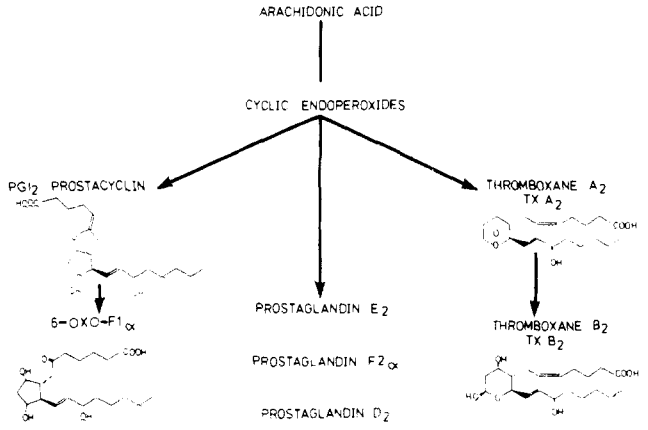

Figure 1.

The measurement of the cutaneous "bleeding time" in animals and humans gives an indication of platelet–vessel wall interactions. In humans, we have demonstrated that a low single dose of aspirin (0.3 g) increases bleeding time, while a larger dose has no effect. These results have been confirmed by others. Moreover, after a single high dose of aspirin (3.9 g), platelet aggregation and TXA_2 formation is blocked 2 h after aspirin and slowly recovers toward pretreatment levels over a period of 168 h. The bleeding time remains unchanged for the first 2 h, but 24 and 72 h after aspirin it is increased and slowly recovers toward pretreatment levels over a period of 168 h, in a manner which mirrors the recovery of TXA_2 formation and platelet aggregability. All these results clearly demonstrate that the balance between TXA_2 and PGI_2 is an important mechanism of control of platelet aggregability in vivo and suggest that aspirin should be given as a small daily dose or even at less frequent intervals. Recently, it has been demonstrated that a single low daily dose of aspirin (160 mg) is effective in preventing thrombosis in patients with surgically implanted arteriovenous shunts (for reviews see ref 3 and 6).

We have also suggested that drugs which effectively inhibit TXA_2 formation will have a superior antithrombotic effect to aspirin. Several of these drugs are now being synthesized and could be ready for testing in humans within the next few years (see ref 3).

Prostacyclin inhibits platelet aggregation by stimulating adenylate cyclase, leading to an increase in cAMP levels in the platelets.[7,8] In this respect prostacyclin is much more potent than either PGE_1 or PGD_2. In contrast to prostacyclin, prostaglandin endoperoxides and thromboxane A_2 reduce cAMP activity in platelets. Because of these opposite effects, we and others have suggested that a balance between TXA_2 and prostacyclin formation regulates platelet cAMP in vivo and therefore platelet aggregability. This proposition has been reinforced by the finding that prostacyclin is a circulating hormone.[9,10] Unlike other prostaglandins such as PGE_2 and $PGF_{2\alpha}$, prostacyclin is not inactivated on passage through the pulmonary circulation.[11] Indeed, the lungs constantly release small amounts of prostacyclin into the passing blood, perhaps from the huge mass of endothelial cells present. The concentration of prostacyclin is higher in arterial than in venous blood for there is about 50% overall inactivation in one circulation through peripheral tissues. This difference, originally obtained in experimental animals, has now been confirmed in human subjects.[12]

Platelets, therefore, may be constantly stimulated by circulating prostacyclin and, consequently, they may have higher cAMP levels and be less aggregable than has ever been detected by in vitro measurements, which are only made after a 10–30 min delay during which the blood is processed. In this period, prostacyclin and its effects will decay. Prostacyclin, therefore, protects the vessel wall against deposition of platelet aggregates, and its discovery provides at least a partial explanation of the long-recognized fact that contact with healthy vascular endothelium is not a stimulus for platelet clumping.

Vascular damage leads to platelet adhesion. The degree of injury is an important determinant, and there is general agreement that, for the development of thrombosis, severe damage or physical detachment of the endothelium must occur. These observations are in accordance with the distribution of prostacyclin synthetase, for it is abundant in the intima and progressively decreases in concentration from the intima to the adventitia. Moreover, the proaggregating elements increase from the subendothelium to the adventitia. These two opposing tendencies render the endothelial lining antiaggregatory and the outer layers of the vessel wall much more thrombogenic.[13]

Prostacyclin inhibits aggregation (platelet–platelet interaction) at much lower concentrations than those needed to inhibit adhesion (platelet–collagen interaction), suggesting that prostacyclin allows platelets to stick to damaged vascular tissue and interact with it, while at the same time preventing or limiting thrombus formation. This process of platelet–vessel wall interaction might be important for vessel repair and regeneration.[3]

Selective inhibition of prostacyclin formation by lipid peroxides (known to be potent inhibitors of prostacyclin synthetase) could lead to a condition in which platelet aggregation is increased, and this could play a role in the development of atherosclerosis. Indeed, lipid peroxidation takes place in plasma as a nonenzymatic reaction and it is known to occur in certain pathological conditions. Hence, lipid peroxides present in these conditions could be shifting the balance of the system in favor of TXA_2 and may predispose to thrombus formation. In this context it is interesting that it has been shown that there is a strong reduction in prostacyclin formation by the heart or vessel walls of rabbits made atherosclerotic. Similarly, human atherosclerotic tissue does not produce prostacyclin, whereas tissue obtained from a nearly normal vessel does.[3]

The role of lipid peroxides in the development of atherosclerosis has been debated for the last 25 years since Glavind and collaborators[14] described the presence of lipid peroxides in human atherosclerotic aortas. They found the peroxide content in diseased arteries to be directly proportional to the severity of the atherosclerosis. Subsequent investigations suggested that Glavind's findings

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were artifactually ascribing the presence of lipid peroxides to their formation during the preparative procedure. Recent observations have given support to Glavind's work favoring the suggestion that lipid peroxides are present in atherosclerotic plaques. Whether or not these peroxides act by inhibiting prostacyclin formation and as a consequence reduce the wall's defense mechanism is not clear, but the theory is of interest, especially since other substances related to atherosclerosis such as the cholesterol carriers, low-density lipoproteins (LDL), have also been shown to inhibit prostacyclin formation in endothelial cell cultures.[15]

Despite the fact that the discovery of the PGI_2/TXA_2 balance is recent, there are already descriptions of clinical conditions in which the balance might be disturbed. Increased production of TXA_2 in vitro by platelets has been found in patients with arterial thrombosis or recurrent venous thrombosis. These conditions are associated with a shortened platelet survival time.[16] In addition, increased sensitivity to aggregating agents and increased release of TXA_2-like activity have been described in rabbits made atherosclerotic by diet and in patients who have survived myocardial infarction.[17] Increased levels of TXB_2 (a stable metabolite of TXA_2) have also been observed in patients with angina during attacks. Moreover, platelets from rats made diabetic release more TXA_2 and their vessel wall produces less prostacyclin, and there is a report that the same might be the case in humans. Other diseases associated with changes in prostacyclin production include uremia, where the hemostatic defect occuring in uremic patients may be attributable to increased prostacyclin production.[18] On the other hand, a lack of prostacyclin production has been suggested in patients with thrombotic thrombocytopenic purpura.[19] Both diseases are linked by the accumulation during uremia, or the lack of production during thrombotic thrombocytopenic purpura, of an ill-defined "plasma factor" which stimulates prostacyclin synthesis. Finally, increased prostacyclin production has been described in blood vessels of the spontaneously hypertensive rat.

As yet, a clear relationship between different diseases and the PGI_2/TXA_2 balance is not established. However, it seems that conditions which favor the development of thrombosis are associated with an increase in TXA_2 and a decrease in prostacyclin formation, whereas an increased prostacyclin formation plus decreased TXA_2 is present in some conditions associated with an increased bleeding tendency.

Prostacyclin or chemical analogues may find a use as a "hormone replacement" therapy in conditions in which excessive platelet aggregation takes place in the circulation, such as acute myocardial infarction or "crescendo angina"; it might also be useful in deep vein thrombosis, different types of shock, disseminated intravascular coagulation, and organ transplantation. Moreover, we have suggested its use in extracorporeal circulation systems, such as cardiopulmonary bypass and renal dialysis. In these systems the main problems are platelet loss with the formation of microaggregates which, when returning to the patient, are responsible for the cerebral and renal impairment observed after bypass. In addition, there are side effects associated with the chronic use of heparin, especially the development of osteoporosis (see ref 3).

Several antiplatelet drugs have been tested in these two situations and some have been used with moderate success. PGE_1 has been reported to be beneficial during cardiopulmonary bypass. However, prostaglandins of the E type induce diarrhea, an effect not shared by prostacyclin. Therefore prostacyclin is not only more potent but more specific in achieving platelet protection. Prostacyclin has now been beneficially used in several systems of extracorporeal circulation in experimental animals, including renal dialysis, cardiopulmonary bypass, and charcoal hemoperfusion. In one of these systems (renal dialysis), prostacyclin can replace heparin altogether. In charcoal hemoperfusion, heparin is also necessary since charcoal particles seem to activate directly the clotting cascade.[20-23]

Following reports that PGE_1 has been used successfully in the treatment of peripheral vascular disease, prostacyclin has been shown to have a similar effect, producing a long-lasting increase in muscle blood flow, disappearance of ischemic pain, and healing of trophic ulcers after an intraarterial infusion to the affected limb for 3 days.[24]

The mechanism of action of antithrombotic drugs which act on platelets has been largely based on their ability to inhibit platelet cyclo-oxygenase, the foremost example being aspirin. A newer approach would be represented by the thromboxane synthetase inhibitors, which might have a superior antithrombotic effect as discussed above. However, the arachidonic acid pathway of platelet aggregation is only one of at least three possible types of aggregation, the other two being the thrombin and the ADP pathways. These are not affected by aspirin-like drugs. The three pathways of aggregation, however, are affected by substances which increase cAMP in the platelets either by stimulating the enzyme (adenylcyclase) which induces this increase, as do PGE_1, PGD_2, and prostacyclin, or by inhibiting the enzyme which degrades it, the 3′,5′-phosphodiesterase, dipyridamole being an example of this group.

So far, prostacyclin is the most potent and comprehensive inhibitor of all forms of aggregation. This fact, together with the endogenous nature of prostacyclin, clearly suggests that the future of antithrombotic therapy lies on the development of compounds with a "prostacyclin" type of action, long acting, orally active, and probably free of the cardiovascular effects of prostacyclin.

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