PROGRESS IN CEREBROVASCULAR DISEASE

Biology and Therapeutic Potential of Prostacyclin

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SEVERAL DISCOVERIES IN THE FIELD OF PROSTAGLANDINS have added substantially to our knowledge of platelet-vessel wall interactions and opened up exciting new therapeutic possibilities. This review will discuss mainly the way in which some of these compounds interact to regulate platelet aggregability in physiological and pathological conditions. The biological properties of prostacyclin and the way in which these might explain its therapeutic potential will be discussed.1

Thromboxane A2 and Prostacyclin

Prostacyclin and thromboxane A2 (TXA2; for structures see figure 1) are both derived from arachidonic acid, a fatty acid present in the phospholipids of cell membranes. Thromboxane A2 is an unstable (t1/2 = 30 seconds at 37°C), powerful vasoconstrictor agent generated by platelets. Prostacyclin is also unstable (t1/2 = 3 minutes at 37°C) but it induces vasodilatation and inhibits platelet aggregation.1

It has been our contention since the discovery of prostacyclin2 that thromboxane A2 and prostacyclin represent the opposite poles of the same homeostatic mechanisms for regulation of platelet aggregability in vivo. Evidence to substantiate this hypothesis has been building up in the last 5 or 6 years.

Prostacyclin-biological Properties

Prostacyclin is the main product of arachidonic acid in all vascular tissues so far tested including human tissue. It is a strong hypotensive agent and a vasodilator of all vascular beds studied including the cerebral circulation (see below).

The ability of the large vessel wall to synthesize prostacyclin is greatest at the intimal surface and progressively decreases toward the adventitia.3 Production of prostacyclin by cultured cells from vessel walls also shows that endothelial cells are the most active producers of prostacyclin.4,5

Using fresh human vascular tissue, we did not find any difference between the rate of production of prostacyclin in vitro by veins and arteries,6 but in rabbits, rats and dogs arteries produce more prostacyclin than veins.7,9 Cultured cells obtained from human pulmonary arteries produce more prostacyclin than those obtained from pulmonary veins.10 In “arterialized” venous (carotid to jugular) grafts implanted in dogs for up to 6 weeks the venous tissue, although becoming arterialized from a structural point of view, maintained a lower production of prostacyclin than the carotid artery.9

Prostacyclin is the most potent endogenous inhibitor of platelet aggregation yet discovered. This effect is short-lasting in vivo, disappearing within 30 minutes of administration. Prostacyclin disaggregates platelets in vitro11 and in the circulation of man.12 Moreover, it inhibits thrombus formation in a coronary artery model in the dog when given locally or systemically,13 protects against sudden death (thought to be due to platelet clumping) induced by intravenous arachidonic acid in rabbits14 and inhibits platelet aggregation in pial vessels of the mouse when applied locally.15

In blood at 37°C, the activity of prostacyclin (as measured by bioassay on vascular smooth muscle) has a half-life of 3 min.16,17 Alkaline pH increases the stability of prostacyclin18,19 so that at pH 10.5 at 25°C, it has a half-life of 100 h. It is stabilised as a pharmaceutical preparation by freeze drying and can be reconstituted for use in man in an alkaline glycerine buffer.

Prostacyclin inhibits platelet aggregation by stimulating adenylate cyclase, leading to an increase in cAMP levels in the platelets.20,21 In this respect prostacyclin is much more potent than either PGE1 or PGD2 and its effect is longer-lasting.

In contrast to TXA2, prostacyclin enhances Ca++ sequestration.22 Moreover, inhibitory effects on platelet phospholipase23,24 and platelet cyclooxygenase25 have been described. All these effects are related to its ability to increase cAMP in platelets. Prostacyclin, by inhibiting several steps in the activation of the arachidonic acid metabolic cascade, exerts an overall control of platelet aggregability in vivo.

Prostacyclin increases cAMP in cells other than platelets.1 Thus, there is the possibility that in these cells an interaction with the thromboxane system could lead to a similar control of cell behaviour to that observed in platelets, suggesting that the prostacyclin/ TXA2 system has wider biological significance in cell regulation. Indeed, prostacyclin inhibits white cell adherence to the vessel wall,26,27 to nylon fibres and to endothelial monolayers in vitro.28 It has recently been shown that prostacyclin increases cAMP in the endothelial cell itself and the authors have suggested that this may act as a negative feedback control for prostacyclin production by the endothelium.29,30

One of the functional characteristics of the intact vascular endothelium is its non-reactivity to platelets. Prostacyclin generation could be responsible for some of the thromboresistant properties of vascular endothelium. Some authors have demonstrated that inhibition

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of prostacyclin production by aspirin does not promote platelet adhesion to the endothelial lining of the rabbit aorta in vivo or in vitro. In addition, neither treatment of vascular endothelium with aspirin or indomethacin nor increasing prostacyclin production by arachidonic acid affected basal platelet adherence. However, in transformed vascular endothelial cells (obtained after viral infection) there was very little prostacyclin generation and platelet adherence was greatly increased. This could be partially reversed by adding exogenous prostacyclin. On the other hand Czervionke and coworkers using washed preparations of labelled human platelets did not observe an effect on platelet adhesion to human endothelial cultures. Platelet adherence in the presence of thrombin increases after treatment with aspirin. This increase was paralleled by a decrease in 6-oxo-PGF\(_2\alpha\) formation and could be reversed by addition of exogenous prostacyclin. In vivo, and in vitro, Baumgartner and Muggli and Tschopp and Baumgartner have shown that aspirin treatment does not enhance platelet adherence to the vascular wall, although after the removal of the vascular endothelium aspirin treatment enhances both adherence and aggregation. Interestingly, they studied vascular tissue from rats, rabbits and guinea pigs and found a decreasing ability to generate prostacyclin from rats to guinea pigs. Moreover, there was a negative correlation between the ability of the vascular tissue of a species to produce prostacyclin and the degree of platelet adherence-aggregation that was observed after interaction of the de-endothelialised vascular tissue with the animals' blood.

The fact that prostacyclin inhibits platelet aggregation (platelet-platelet interaction) at much lower concentrations than those needed to inhibit adhesion (platelet-collagen interaction), suggests that prostacyclin allows platelets to stick to vascular tissue and to interact with it, while at the same time preventing or limiting thrombus formation. Thus prostacyclin need not impair the well-recognised ability of platelets to participate in the repair of a damaged vessel wall. In addition, platelets adhering to a site where prostacyclin synthetase is present could well feed the enzyme with endoperoxide, thereby producing prostacyclin and preventing other platelets from clumping onto the adhering platelets, limiting the cells to a monolayer.

It has also been demonstrated that shortly after balloon de-endothelialisation of the aortae of rabbits there is a closely adherent layer of spread platelets. A small reduction of adherent platelets could be observed in animals receiving prostacyclin at 50–100 ng/kg/min. Only concentrations of 650–850 ng/kg/min could inhibit this platelet adhesion. Eldor et al. have demonstrated that the vascular endothelium is the only source of prostacyclin generated in the luminal surface of a rabbit aorta. Moreover, after balloon catheter de-endothelialisation the capacity for generation of prostacyclin is abolished and only recovers slowly over a period of 70 days, concomitant with the appearance of neo-intimal cells on the vessel surface. The authors also observed in the de-endothelialised areas a "carpet of platelets" which slowly disappeared during the time of re-endothelialisation.

All this work suggests that prostacyclin, although not responsible for all the thromboresistant properties of vascular endothelium, plays a very important part in the control of the deposition of platelet aggregates.

**Prostacyclin and Thromboxane Balance. Aspirin and TXA\(_2\) Synthetase Inhibitors**

Aspirin binds covalently to the active site of cyclooxygenase and therefore inhibits the enzyme in platelets for their entire lifespan because platelets are unable to synthesise new protein. Aspirin also has an effect on the platelet precursors in the marrow. Thus, a single therapeutic dose of aspirin will lead to a platelet defect that lasts for well over a week. This long-lasting effect is observed only with aspirin and not with other commonly used aspirin-like drugs, which have a shorter inhibitory effect. This consideration has encouraged clinical trials in which aspirin has been used to prevent thrombotic phenomena. So far, however, the evidence in favour of this clinical use of aspirin is not very satisfactory and the reasons are becoming clear as research progresses. A consequence of the discovery of prostacyclin has been the need to re-examine the use of aspirin as an antithrombotic compound. Two important considerations have emerged in relation to aspirin:
1) inhibition of the vascular cyclo-oxygenase, unlike the platelet cyclo-oxygenase, may persist for a much shorter period because of the generation of new enzyme; and 2) the platelet cyclo-oxygenase seems to be more sensitive in vitro and in vivo than the vessel wall cyclo-oxygenase to the inhibitory action of aspirin, although reports to the contrary have been published. During the past 3 to 4 years, an attempt has been made to find a low dose of aspirin that will achieve inhibition of TXA₂ formation in man without affecting prostacyclin production. The available data suggest that it will be extremely difficult to find a suitable dose or schedule of aspirin in man that will achieve selective and long-lasting inhibition of TXA₂. However, one recent study by Patrignani et al. suggests that a daily dose of 0.45 mg/kg given for seven days to volunteers produces an accumulative and complete inhibition of \( \text{TXB}_2 \) production by platelets in vivo, without significantly reducing the urinary excretion of 6-oxo-PGF₁₀₂ (the degradation product of prostacyclin). The significance of this finding is not clear since the origin of urinary 6-oxo-PGF₁₀₂ is not known, a fact recognised by these authors.

The main difficulty with using aspirin as an anti-thrombotic agent relates to the fact that even if a suitable dose (which inhibits only thromboxane A₂ formation) is found it will probably not prove to be a better anti-thrombotic drug than it has already been shown in the few trials in which it has been slightly efficacious. The reason is that platelet aggregation is a complex mechanism that takes place via different 'pathways', the generation and actions of TXA₂ being only one of them (the other two identified at present are the ADP and thrombin pathways).

Only the stimuli that induce platelet aggregation via the release of TXA₂ will be affected by aspirin treatment, the others being largely unaffected. Since not enough is known about the pathophysiology of intravascular thrombosis it is difficult to predict what to expect after aspirin treatment. It is highly likely that platelet aggregation during disseminated intravascular coagulation, venous thrombosis and arterial thrombosis have different triggering mechanisms, and it is also possible that platelet aggregation on a fissure of an atherosclerotic plaque or during coronary vasospasm might depend on the activation of different pathways.

A selective inhibitor of thromboxane synthetase might be a superior anti-thrombotic agent to aspirin by allowing prostacyclin formation by vessel walls or other cells either from their own endoperoxides or from those released from platelets. Working on selective thromboxane synthetase inhibitors is beginning to appear, including the first publications on administration of one of these compounds to humans. In two experimental studies, it has now been shown that TXA₂ synthetase inhibitors have a superior anti-thrombotic action to aspirin.

**Prostacyclin and TXA₂ In Disease**

A number of diseases have now been related to an imbalance in the prostacyclin/TXA₂ system. Platelets from patients with arterial thrombosis, deep venous thrombosis, or recurrent venous thrombosis produce more PG endoperoxides and TXA₂ than normal and have a shortened survival time. Platelets from rabbits made atherosclerotic by dietary manipulation and from patients who have survived myocardial infarction are abnormally sensitive to aggregating agents and produce more TXA₂ than controls. Elevated \( \text{TXB}_2 \) levels have been demonstrated in the blood of patients with Prinzmetal's angina and vasotonic angina. Hirsh and colleagues also studied \( \text{TXB}_2 \) levels in coronary sinus blood of patients with unstable angina. They concluded that local thromboxane A₂ release is associated with recent episodes of angina but were unable to distinguish whether the release was cause or effect.

Platelets from rats made diabetic release more TXA₂ than normal, whereas their blood vessels show a reduced production of prostacyclin; these effects are reversed by chronic insulin treatment. Prostacyclin production by blood vessels from patients with diabetes is depressed and circulating levels of 6-oxo-PGF₁₀₂ are reduced in diabetic patients with proliferative retinopathy. Davie and colleagues have confirmed that vessels taken from diabetic patients produced less prostacyclin than from normal subjects. However, their results did not support an association between reduced prostacyclin production and diabetic retinopathy.

Thrombocytopenic purpura (TTP), like diabetes, is associated with formation of microvascular thromboemboli, and a deficiency in prostacyclin production may be responsible for the increased platelet consumption which occurs in TTP. This deficiency is postulated to be secondary to a lack of a 'plasma factor' which normally stimulates prostacyclin production. A patient with TTP had an undetectable level of 6-oxo-PGF₁₀₂ (<60 pg/ml) whereas the mean value in control subjects was 154 ± 48 pg/ml. It has been postulated that a deficiency or lack of maturation of prostacyclin synthetase, when combined with elevated levels of endoperoxides and TXA₂, may account for sudden infant death but there is no experimental evidence to support this hypothesis. Prostacyclin production is significantly lower in umbilical and placental vessels from pre-eclamptic patients than in those from normally pregnant women. An increased prostacyclin production, resulting from an accumulation of the 'plasma factor' which stimulates prostacyclin synthesis, has been suggested to explain the haemostatic defect in uremic patients. Patients with Barter's syndrome excrete in the urine about four times as much 6-oxo-PGF₁₀₂ as controls. This has led to the suggestion that overproduction of prostacyclin mediates both the hyper-reninaemia and the hyporesponsiveness to pressor agents observed in these patients. Finally, enhanced prostacyclin production by blood vessels of spontaneously hypertensive rats has been demonstrated. However, Grose and colleagues have described a diminished excretion of 6-oxo-PGF₁₀₂ in the urine of patients with essential hypertension. This could reflect diminished prostacyclin production by the kidney itself, or less likely, by
the body as a whole. Thromboxane A₂ produced during ligation of the coronary artery of the dog produces arrhythmias and also vasoconstriction induced by TXA₂ in the gastric mucosa of the dog produces gastric ulceration. During transplant rejection there is an increased level of TXB₂ excreted in the urine preceding the acute crisis and also, high levels of TXB₂ have been found in the venous blood of patients suffering from endotoxin shock.

**Prostacyclin and Atherosclerosis**

It has been shown that 15-HPAA, a lipid peroxide, is a potent (IC₅₀ 0.48 µg/ml) and selective inhibitor of prostacyclin generation by vessel wall microsomes or by fresh vascular tissue. Other fatty acid peroxides and their methyl esters behave similarly. High concentrations of lipid peroxides have been demonstrated in advanced atherosclerotic lesions. Lipid peroxidation induced by free radical formation is known to occur in vitamin E deficiency, the ageing process and perhaps also in hyperlipidaemia accompanying atherosclerosis. Accumulation of lipid peroxides in atheromatous plaques could predispose to thrombus formation by inhibiting generation of prostacyclin by the vessel wall without affecting thromboxane A₂ production by platelets. Moreover, platelet aggregation is induced by 15-HPAA and this aggregation is not inhibited by adenosine or PGE₁. D’Angelo and co-workers reported that human atheromatous plaques from three patients were incapable of prostacyclin production. Prostacyclin generation by atherosclerotic arterial tissue has been shown to be significantly lower than from normal arterial tissue but no difference was found between early and advanced atherosclerotic lesions. This suggests that the early “fatty streak” may be a biochemically critical stage of the atherosclerotic process. In rabbits whose aortae have been de-endothelialised using the balloon catheter technique, the production of prostacyclin in the luminal surface of the vessel is reduced and recovers slowly to “normal levels” with re-endothelialisation over a period of about 70 days. The recovery of prostacyclin formation does not occur in rabbits made hypercholesterolemic by diet.

The same group of authors have also suggested that the accumulation of cholesteryl esters in the areas of damage of the vessel wall could be related to the decrease in prostacyclin synthesis since prostacyclin was demonstrated to enhance acid cholesteryl ester hydrolase activity in cultured vascular smooth muscle and to a decrease in prostacyclin production.

All these results, therefore, suggest that it would be worth exploring whether attempts to reduce lipid peroxide formation by inhibiting peroxidation influence the development of atherosclerosis and arterial thrombosis. Vitamin E acts as an antioxidant and perhaps its empirical use in arterial disease in the past had in fact a biochemical rationale. It is important to point out that it has been shown in vitro that human diploid fibroblasts which produce prostacyclin lose the ability to do so during ageing while the production of other arachidonic acid metabolites like PGE₂, PGF₂α and thromboxane A₂ increases. In addition aortic smooth muscle cells obtained from old rats produce less prostacyclin in culture than those obtained from young animals. This is due to a specific decrease in the prostacyclin synthetase activity since the cyclooxygenase activity was similar in both groups. Similar results have been obtained with fresh swine arteries and in vitro using bovine smooth muscle and endothelial cells it has been observed that during subculture the ability to generate prostacyclin decreases while PGE₂ formation increases. Whether these changes are due to a specific damage of the prostacyclin synthetase due to increased lipid peroxidation with age remains to be investigated.

Raised concentrations of low density lipoprotein (LDL) are regarded as one of the risk factors associated with ischaemic heart disease whereas high density lipoprotein (HDL) is thought to protect against the disease. Nordoy and co-workers were the first to show that LDL reduced the release of a prostacyclin-like substance by human endothelial cells. Beitz and Förster extended these observations by showing that LDL inhibited, whereas HDL stimulated prostacyclin synthesis. A mixture of low LDL and high HDL also stimulated, prostacyclin synthesis. Gryglewski and Szczeklik have confirmed that LDL inhibits prostacyclin synthesis. They also analysed lipoproteins taken from a group of hyperlipidaemics and found the LDL fraction (but not the HDL) contained lipid peroxides at a concentration several times higher than those in the total serum. Thus, the interesting possibility arises that it is the lipid peroxide associated with LDL which inhibits prostacyclin synthesis.

Cell proliferation in vitro is inhibited by substances which stimulate cyclic AMP formation. Cell growth in tissue culture, including vascular smooth muscle cell culture, is inhibited by PGE₁. Possibly prostacyclin has a role in the regulation of cell growth in the vascular wall. Smooth muscle proliferation in atherosclerotic plaques might be a consequence of inhibition of prostacyclin generation by lipid peroxides.

**Modification of Fatty Acid Precursors**

Eicosapentaenoic acid (EPA) is a polyunsaturated fatty acid like arachidonic acid but has a higher degree of unsaturation. It gives rise to prostaglandins of the ‘3’ series and when incubated with vascular tissue leads to the release of an antiaggregating substance. This effect persists after subculture. In addition, it has been shown in rats that a vitamin E deficient diet leads to an increase in peroxide levels in the aortae and to a decrease in prostacyclin production in vitro. All these results, therefore, suggest that it would be worth exploring whether attempts to reduce lipid peroxide formation by inhibiting peroxidation influence the development of atherosclerosis and arterial thrombosis. Vitamin E acts as an antioxidant and perhaps its empirical use in arterial disease in the past had in fact a biochemical rationale. It is important to point out that it has been shown in vitro that human diploid fibroblasts which produce prostacyclin lose the ability to do so during ageing while the production of other arachidonic acid metabolites like PGE₂, PGF₂α and thromboxane A₂ increases. In addition aortic smooth muscle cells obtained from old rats produce less prostacyclin in culture than those obtained from young animals. This is due to a specific decrease in the prostacyclin synthetase activity since the cyclooxygenase activity was similar in both groups. Similar results have been obtained with fresh swine arteries and in vitro using bovine smooth muscle and endothelial cells it has been observed that during subculture the ability to generate prostacyclin decreases while PGE₂ formation increases. Whether these changes are due to a specific damage of the prostacyclin synthetase due to increased lipid peroxidation with age remains to be investigated.

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Synthetic Δ17 prostacyclin or PGH₂, is as potent an anti-aggregating agent as prostacyclin. In contrast, thromboxane A₂ has a weaker pro-aggregating activity than TXA₂. The fatty acid available for PG biosynthesis in Greenland Eskimos is mainly EPA, unlike that in Caucasians which is mainly arachidonic acid. These differences may explain why Eskimos have a lower incidence of acute myocardial infarction, low blood cholesterol levels, and an increased tendency to bleed. This prolonged bleeding time is related to a reduction in ex vivo platelet aggregability. The plasma concentrations of cholesterol, triglyceride, low and very low density lipoprotein (VLDL) are low in Eskimos, whereas that of high density lipoprotein is high.

Eicosapentaenoic acid inhibits platelet aggregation in platelet rich plasma stimulated by ADP, collagen, arachidonic acid, and a synthetic analogue of PGH₂. Also, EPA inhibits aggregation in aspirin and indomethacin-treated platelets and inhibits thrombin-induced aggregation. It is clear, therefore, that both prostaglandin-dependent and independent pathways of platelet aggregation are inhibited by EPA in vitro. In vivo, however, EPA would be incorporated into platelet phospholipids, to some extent replacing arachidonic acid and exerting an antithrombotic effect either by competing with remaining arachidonic acid for cyclooxygenase and lipoxygenase or by being converted to the less pro-aggregatory PGH₃. Studying seven Caucasians who had been on a mackerel diet for 1 week, Seiss and colleagues showed a reduced sensitivity of platelets to collagen, associated with a reduced ability to produce thromboxane B₂, which was dependent on the ratio of C20:5/C20:4 in platelet phospholipids. ADP-induced aggregation was significantly reduced in some subjects and platelet aggregation to exogenously added arachidonic acid was unchanged, indicating normal cyclo-oxygenase activity. Similarly, Sanders and co-workers showed a significant increase in bleeding time of 40% in volunteers who had taken cod liver oil (equivalent to 1.8 g eicosapentaenoic acid) daily for six weeks. This was consistent with a decrease in arachidonic acid and an increase in eicosapentaenoic acid in the platelet phospholipids. This diet also led after 6 weeks to a reduction in TXB₂ concentrations of cholesterol, triglyceride, low and very low density lipoprotein (VLDL) are low in Eskimos, whereas that of high density lipoprotein is high.

Cerebrovascular Effects of Prostacyclin and Thromboxane A₂

Prostacyclin is generated by brain blood vessels of all species so far studied including baboon and human cerebral arteries in vitro. Much lower amounts are generated by grey and white matter and this might be a reflection of the synthesis by arterioles and capillaries. Synthesis of prostacyclin by rat and bovine arterioles and capillaries has been demonstrated by Goehlert et al. and Maurer et al. The choroid plexus also has a high prostacyclin synthesizing capacity.

The high prostacyclin synthesizing capacity of the cerebral vessels suggests that prostacyclin may be involved in the regulation of blood flow in the brain. Indeed, several authors have demonstrated that treatment with indomethacin reduces cerebral blood flow in several species including man, although not all authors are in agreement (for review see). Thromboxane A₂ is a constrictor of cat isolated cerebral arteries and human basilar artery strips. In human cerebral artery strips it is a more potent constrictor than 5-hydroxytryptamine.

Prostacyclin, on the other hand, at low concentrations (≈ 10⁻⁸ M) relaxes canine cerebral and basilar arteries, baboon cerebral and human cerebral, basilar and pial vessels in vitro although there is one report describing contractile effects. In pial human vessels prostacyclin strongly antagonises the contractions induced by several vasoactive substances and by cerebrospinal fluid.

In vivo, prostacyclin given locally induces vasodilation in the cerebral arterioles of the cat and given systemically in the baboon it induces a prolonged dilatation of the cerebral circulation.

In the baboon, the intracarotid infusion of prostacyclin not only increases cerebral blood flow to up to 70% but also reverses the vasoconstrictor effect of indomethacin during hypercapnia. Prostacyclin administered intravenously in cats also reverses vasoconstriction induced by application of oxyhemoglobin.

Interestingly, it has been demonstrated using a technique of compression-induced ischaemia of the brain of dogs resulting in an impaired reperfusion, that pretreatment of these animals with indomethacin or pretreatment with indomethacin plus an infusion of prostacyclin after ischaemia significantly enhances.
post-ischaemic reperfusion. If indomethacin was given after ischaemia it did not improve reperfusion. Since indomethacin induces a decrease in cerebral blood flow in normal animals, one explanation for these results is that early during ischaemia, arachidonic acid-derived products (probably TXA2) are generated by platelets or other cells and these induce further vasoconstriction. The role of thromboxane A2 in cerebral vasospasm has been suggested before. Therefore the use of a combination of thromboxane synthetase inhibitor plus an infusion of prostacyclin would be of great interest. Recently, a thromboxane synthetase inhibitor has been used in the prevention of cerebral vasospasm after aneurismal rupture and the authors report a positive effect.

A decrease in prostacyclin formation has been reported in the canine basilar arteries of dogs after subarachnoid haemorrhage. Cerebral ischaemia induced in experimental animals leads to an accumulation of lipid peroxides and arachidonic acid metabolites with potent vasoconstrictor properties. The possibility that lipid peroxides, which inhibit prostacyclin formation might play a role during cerebral vasospasm has been considered. This inhibition of prostacyclin formation could be the result of a long term process taking place over a period of years during the development of atherosclerosis (as described above) which is then further potentiated by the inhibition of prostacyclin induced by lipid peroxides generated during the haemorrhagic episode.

So far, there are very few studies on the effect of prostacyclin in human cerebral blood flow. In a preliminary study, it has been shown that 5 ng/kg/min prostacyclin given intravenously to volunteers produces a slight decrease in cerebral blood flow repetition of volunteers but significantly increases it after a reduction induced by administration of indomethacin to the subjects. (Note: Since this review was written, a report on the use of prostacyclin in patients with ischaemic stroke has been published. The authors report a dramatic improvement in 10 patients subjected to intravenous infusion of prostacyclin. Results of double blind studies are now necessary to validate these findings.)

Clinical Applications

a) Extracorporeal Circulations

The assessment of prostacyclin as a therapeutic agent is in its early stages but results from open and double blind clinical trials as well as from individual case reports already indicate the conditions in which prostacyclin might be useful. One such condition occurs when blood is exteriorized and made to circulate through artificial surfaces as in cardiopulmonary bypass operations, charcoal haemoperfusion and renal dialysis. In the course of such procedures thrombocytopenia and loss of platelet function occur and contribute to the well-recognized haemostatic defect which follows charcoal haemoperfusion and prolonged cardiopulmonary bypass in man. Formation of microemboli during cardiopulmonary bypass may also contribute to cerebral complications which sometimes follow this procedure. In animals subjected to experimental renal dialysis, charcoal haemoperfusion and cardiopulmonary bypass infusion of prostacyclin prevented this platelet damage and thrombocytopenia, thus increasing the biocompatibility of the procedure. Platelet aggregates in the blood returning to the animals, as measured by Swank screen filtration pressure, were also reduced by infusion of prostacyclin. These findings have been confirmed in patients with fulminant hepatic failure undergoing charcoal haemoperfusion. Prostacyclin infusion prevented the fall in platelet count and elevation of beta-thromboglobulin seen in the control patients. In addition, two of the control patients developed marked hypotension during the procedure, in one associated with a marked rise in Swank screen filtration pressure, while this did not occur in the prostacyclin-treated patients. Gimson et al. have carried out 198 serial charcoal haemoperfusions in 76 patients with fulminant hepatic failure. In 31 of those patients which were subjected to haemoperfusion while having signs of Grade III encephalopathy a remarkable survival rate of 65% was observed. In 45 patients with Grade IV encephalopathy 10 showed improvement of consciousness. The overall survival for the two groups was 38%. The authors report an excellent biocompatibility of the system in the presence of prostacyclin, which explains the success of the procedure and the possibility of treating this type of patient at an early stage of the condition.

Several double blind clinical trials of prostacyclin in cardiopulmonary bypass have been published. The treatment groups showed a preservation of platelet number and function, with a reduction in the blood loss in the first 18 hours after operation. In the trial by Longmore et al. the blood loss was significantly lower than in the control group.

The observation that prostacyclin potentiates the effects of heparin led to further studies on this interaction. These demonstrated that prostacyclin has a small indirect anticoagulant effect. Indeed, platelets stimulated by low doses of aggregating agents accelerate clotting by providing a surface upon which coagulation factors can combine and react more efficiently. Prostacyclin, by preventing platelet activation, inhibits the shortening of clotting time produced when either kaolin or collagen is incubated with platelet-rich plasma. Platelets release antithrombin activity, which reduces the anticoagulant effect of heparin in vitro. Prostacyclin, by inhibiting this release and by preventing the development of procoagulant activity, can enhance the action of heparin by as much as one hundred per cent.

Heparin therapy in some patients is complicated by thrombocytopenia and thrombo-embolic episodes and in vitro heparin can cause platelet aggregation and potentiate aggregation caused by other aggregating agents. Perhaps because the stimulus to coagulation is milder, we showed that during haemodialysis in dogs, prostacyclin could be used alone to prevent platelet loss and coagulation. Heparin was not
needed. This surprising result has now been confirmed by several groups. In particular, Zusman et al.\textsuperscript{161} and Turney et al.\textsuperscript{162} infused prostacyclin intravenously before dialysis and into the arterial line during dialysis. Prostacyclin safely replaced heparin as the sole antithrombotic agent during haemodialysis and could well be more advantageous when anticoagulation is contraindicated. Indeed, Turney & Weston\textsuperscript{163} have safely used prostacyclin instead of heparin during dialysis in more than 50 patients.

It is clear that the beneficial effects of prostacyclin in extracorporeal circulations depend upon its anti-platelet activity.

b) Other Applications

Szczeklik et al.\textsuperscript{164} reported striking and prolonged benefits following intraarterial infusion of prostacyclin in five patients with advanced atherosclerotic lower limb peripheral vascular disease. Other reports also suggest that prostacyclin may have beneficial effects in peripheral artery disease.\textsuperscript{165,166} However, the results of double blind trials now in progress are necessary before a full evaluation of the efficacy of prostacyclin in this condition can be made. Zygulska-Mach et al.\textsuperscript{167} infused prostacyclin into three patients with sudden blockage of central retinal veins. Improvement was observed in those two patients who were treated within the first 48 hours.

Prostacyclin also induces significant and long-lasting improvements in Raynaud's phenomenon. Intravenous infusion of the drug for 72 hours at the maximum tolerated dose (up to 10 ng/kg/min) produced striking reductions in the frequency, duration and severity of the disease in 21 of 24 patients. In all patients who responded, the improvement lasted for weeks (mean 9–10 weeks) and in 3 patients, subjective improvement was still reported 6 months after the infusion. Pain relief was a striking feature, presumably associated with the increased blood flow indicated by increased temperature of the hands and fingers. Although this was an open trial, the authors' previous experience showed that there was no placebo response to saline infusion.\textsuperscript{168}

It is difficult to ascribe these long term benefits of prostacyclin either to the anti-platelet activity or to the direct vasodilator action, which are normally over within 20–40 minutes of stopping administration. Dowd et al.\textsuperscript{168} suggested that modulation by prostacyclin of immunological phenomena and alteration of neutrophil function may be the basis of the long-lasting effects of such an ephemeral substance.

Prostacyclin has been successfully used in cases of pulmonary hypertension.\textsuperscript{169} In a group of patients with pulmonary hypertension secondary to mitral valve stenosis, prostacyclin caused a dose-dependent pulmonary vasodilatation with no observed side effects.\textsuperscript{170} In both of the above studies prostacyclin was shown to be more effective than PGE\textsubscript{1}. Single case studies have suggested that prostacyclin may be useful in the treatment of patient ductus arteriosus\textsuperscript{171} and pre-eclamptic toxaemia.\textsuperscript{172}

Bergman et al.\textsuperscript{173} gave an intravenous infusion of prostacyclin to patients with coronary artery disease and showed that doses of 2–8 ng/kg/min for 10 min had no deleterious effects. Heart rate and cardiac index were increased and mean blood pressure, systemic and pulmonary resistance all fell. Mean atrial pacing time to angina rose from 142 to 241 seconds. They concluded that acute administration of prostacyclin was beneficial in angina, having effects similar to those of the short acting nitrates. Hall & Dewar\textsuperscript{174} concluded from their study of five patients with coronary artery disease that prostacyclin can safely be infused directly into diseased coronary arteries and Szczeklik and Gryglewski\textsuperscript{175} found a beneficial effect of intravenous prostacyclin infusions in patients with unstable angina. However, Chierchia et al.\textsuperscript{176} found intravenous administration of prostacyclin to be without effect on the number, severity and duration of ischaemic episodes in 8 of 9 patients with variant angina. Consistent relief was seen on administration of prostacyclin to the ninth patient.

A prostacyclin deficiency has been reported in thrombotic thrombocytopenic purpura (TTP).\textsuperscript{63} Infusion of prostacyclin into two patients with TTP did not produce an increase in circulating platelet count.\textsuperscript{63,177} However, Fitzgerald et al.\textsuperscript{178} have reported an increase in platelet count and an improvement in the neurological status of one such patient during 18 days of prostacyclin infusion. They were sufficiently encouraged to conclude that the controlled evaluation of prostacyclin in TTP was warranted.

The work of Mundy et al.\textsuperscript{179} showed that infusion of prostacyclin protected transplanted kidneys from hyperimmune rejection in dogs. Leithner et al.\textsuperscript{180} have now shown in 8 patients with chronic renal transplant rejection that intravenous infusion of prostacyclin at 5 ng/kg/min for 5 days resulted in less platelet consumption by the kidneys and an improvement in transplant function.

Clearly, there are many clinical conditions which may respond to prostacyclin treatment and its place (or that of stable analogues) in therapeutics will be defined in the next few years. Some of these conditions are pre-eclamptic toxaemia,\textsuperscript{181} haemolytic uraemic syndrome,\textsuperscript{182} peptic ulceration,\textsuperscript{71} the thrombotic complications associated with transplant rejection,\textsuperscript{179} the prevention of tumour metastasis\textsuperscript{183} and the treatment of pulmonary embolism.\textsuperscript{184}

**Prostacyclin and Cytoprotection**

Recently it has become increasingly apparent that, in addition to its well-known vasodilator and anti-aggregating actions, prostacyclin shares with other prostaglandins a "cytoprotective activity," as yet not clearly defined. This activity has usually been studied on gastric ulcers.\textsuperscript{185} We have suggested\textsuperscript{186} that this third property may be important in explaining certain therapeutic effects of prostacyclin. In some experimental models of myocardial infarction, prostacyclin reduces infarct size\textsuperscript{187,189} and also decreases oxygen demand\textsuperscript{189} and the release of cathepsin D and creatinine phospho-
kinase from infarcted areas. In other studies on the effects of prostacyclin on lung injury in sheep, Demling et al. found that prostacyclin protected the lungs against injury induced by endotoxin. A beneficial effect of prostacyclin has also been reported in endotoxin shock in the dog and in the cat where it improves splanchic blood flow and reduces the formation and release of lysosomal hydrolases (Cathepsin D). The effects of hypoxic damage in the cat isolated perfused liver are also substantially reduced by prostacyclin.

All these effects could be related to an effect observed recently in our laboratory. We found that the addition of prostacyclin during the separation from blood and the subsequent washing of platelets substantially improves their viability in vitro, but more importantly, whereas normal in vitro platelet survival time is about 4 to 8 h, platelets prepared with the aid of prostacyclin remain functional for more than 72 h. This effect was not accompanied by an increase in cAMP level in platelets, thus separating it from the classical anti-aggregating effect. Interestingly, there has been a study demonstrating a dissociation between anti-aggregating and cytoprotective effects of a prostacyclin analogue in a model of acute myocardial ischemia. All these results suggest that some of the therapeutic effects of prostacyclin might be related to this cytoprotective effect and indicate a wider therapeutic use of prostacyclin in cell or tissue preservation in vivo and in vitro. However, more work is needed in this area before a final assessment can be made.

Conclusion

Since its discovery in 1976 prostacyclin now known by the generic name epoprostenol, has been intensively studied, both as an endogenous hormone and as an exogenously administered agent.

Our knowledge of the causes and treatment of cardiovascular diseases will be improved over the next years by the availability of prostacyclin or closely related analogues. Efforts are being made to obtain stable compounds which are easier to use and with fewer cardiovascular effects than prostacyclin itself. If this is achieved, potent, probably orally active analogues will be available for the treatment of cardiovascular and other conditions as well as for further research into the physiology and pathophysiology of platelet-vessel wall interactions; for example the participation of platelets in the development of atheroma will be more accurately evaluated and the possibility that some of the therapeutic actions of prostacyclin are related to its cytoprotective effect will be more fully assessed.

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