Do Platelets Have Anything To Do With Diabetic Microvascular Disease?

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SUMMARY

It has been postulated that abnormal platelet and endothelial function may contribute to microangiopathy in diabetes mellitus. If this proposal is correct, alterations in platelet and endothelial function should be found before the appearance of vascular disease in insulin-dependent patients and in animal models of diabetes mellitus. This appears to be the case for the following: platelet aggregation, increased platelet production of the proaggregatory prostaglandin metabolite thromboxane, decreased endothelial production of the antiaggregatory prostaglandin prostacyclin, and decreased platelet survival. Insulin therapy will return some of these findings to normal. Platelet-plasma interactions that promote platelet aggregation and increased plasma levels of the platelet-specific protein β-thromboglobulin have been reported in insulin-dependent diabetic patients who have not manifested vascular complications as well as in those with vascular complications. It has now been demonstrated in animal models that platelet microthrombi are found in small retinal vessels after months of experimental diabetes.

Collectively, these findings demonstrate that alterations in platelet and endothelial function that favor thrombosis occur early in the diabetic state and may contribute to microvascular disease. There are several ongoing studies of antiplatelet agents in diabetic vascular disease that will provide clinical evidence bearing on the major postulate. Until these and other studies are completed, the platelet-endothelial story remains an attractive hypothesis in the genesis of diabetic microvascular disease. DIABETES *32* (Suppl. 2):14–19, 1983.

For the past 10–15 yr, there has been great interest in the possibility that abnormal platelet and/or endothelial function may contribute to diabetic vascular disease. Abnormalities in platelet and endothelial function have been found in cross-sectional studies of diabetic animals and patients, and cause-and-effect relationships have been postulated. Review articles have considered this in depth.[1–4] However, the association of platelet and endothelial functional abnormalities that would favor platelet deposition and thrombus formation in diabetes can be interpreted in several ways. First, the abnormalities could contribute directly to diabetic vascular disease; second, they could be totally unrelated; third, diabetic vascular disease could cause the defects; and fourth, they could both be contributory to, and result from, the vascular disease.

In this article, we explore the postulate that altered platelet and endothelial function may contribute in some way to diabetic vascular disease. If this were the case, one would expect the following findings:

1. Altered platelet function before the appearance of vascular disease in insulin-dependent diabetic (IDDM) patients and animals. Such abnormalities might include: (a) increased sensitivity of platelets to aggregating materials in vitro; (b) platelet-plasma interactions; (c) increased platelet production of proaggregatory prostaglandins and metabolites; (d) increased plasma levels of a platelet-specific protein, β-thromboglobulin (β-TG); and (e) decreased in vivo platelet survival.
2. Altered endothelial function in uncomplicated IDDM. This might include: (a) decreased production of the antiaggregatory prostaglandin, prostacyclin (PGI_2); (b) increased plasma levels of the endothelial protein, von Willebrand factor (VIII R:WF); and (c) decreased fibrinolysis due to diminished release of plasminogen activator by the endothelium.
3. Improvement in altered platelet and/or endothelial function with insulin therapy of diabetes. This would imply that the defect(s) is related to the insulin-deficient diabetic state.
4. Detection of platelet microthrombi in experimental diabetes.
5. Diminished rate of progression of diabetic vascular dis-

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TABLE 1
Increased platelet aggregation in nonvascular insulin-dependent diabetes mellitus

Findings	Author	Year	Agents	Comments
Positive	Sagel[4]	1975	ADP, epi, coll.	↑ aggregation in nonvascular DM
	Halushka[5,6]	1977, 1981	ADP, epi, coll. AA	↑ PGE_2, ↑ TXB_2
	Stuart[7]	1979	Thrombin	Pregnancy, ↑ MDA
	Silberbauer[8]	1981	ADP, coll.	No effect—retinopathy
	Paulsen[9]	1981	ADP	No retinopathy
	Janka[10]	1981	ADP, coll.	No angiopathy
Negative	Petersen[11]	1978	ADP, epi	53% vascular insulin
	Corbella[12]	1979	ADP, epi	Children

ease with the use of antiplatelet agents in prospective randomized clinical trial.

Studies relating to each of these areas are reviewed.

PLATELET FUNCTION IN NONVASCULAR IDDM

Platelet aggregation. There are many studies of in vitro platelet aggregation in diabetes. Most of these have been cited in a recent review.[1] If platelet aggregation were to contribute to diabetic microvascular disease, one might expect that some abnormality of platelet function would precede the development of vascular disease. It is therefore appropriate to focus on articles in which studies have been reported of platelet aggregation in insulin-dependent diabetic patients who were found to be free from microvascular disease. In such studies, absence of retinopathy by ophthalmologic examination in the majority or all of the patients studied was documented as a major indicator of microvascular status.

There are nine studies that fulfill these criteria (Table 1). In seven of them, increased sensitivity to platelet aggregating agents in vitro was found. A variety of agents was investigated, with general agreement that increased sensitivity to ADP and collagen occurred. In general, however, platelet hypersensitivity has been found to be more prominent in diabetics with established microvascular complications in both these and other published studies. (See refs. 1–3 for reviews.) In a few studies of IDDM without vascular complications, no in vitro sensitivity of platelets to aggregating agents could be found (Table 1).

Some authors have reported that spontaneous in vitro platelet aggregation is present in IDDM patients without microvascular disease[8,10,13,14] (Table 2). Variation in techniques used was present, but generally platelets were stirred in platelet-rich plasma or in whole blood, and spontaneous aggregation of platelets was quantitated within 10 min of starting the test. One study has shown that diabetics with "pre-retinopathy" have a slightly decreased platelet count ratio (PCR), suggesting spontaneous platelet aggregation in vivo or in vitro.[8]

TABLE 2
Spontaneous platelet aggregation in nonvascular insulin-dependent diabetes mellitus

Author	Year	Positive results found
Breddin[13]	1981	38% of IDDM ± retinopathy
Paulsen[9]	1981	39% of IDDM, 68%—no retinopathy
Janka[10]	1981	22% of IDDM, no retinopathy (NS)
Juhan[14]	1982	Increased 3× in whole blood, new IDDM
Silberbauer[8]	1981	Increased platelet count ratio (PCR) in pre-retinopathy

Because clinical studies such as these are extremely difficult to control, many observers have performed studies of platelet aggregation in experimental diabetes. Results from seven studies[15–21] are shown in Table 3. In the rabbit and rat, using alloxan or streptozotocin to induce a diabetic state, increased sensitivity of platelets to aggregating agents may be observed in vivo and in vitro. In several studies, this could be seen only when washed platelets were used,[17,20] suggesting the presence of plasma inhibitors of platelet aggregation. One study found a prolonged aggregation time in cerebral and mesenteric microvessels in vivo in the spontaneously diabetic obese mouse (ob/ob or db^+/db^+).[21] Increased sensitivity of washed platelets to aggregating agents can be demonstrated in some animal models of diabetes in the presumed absence of microvascular disease.

Platelet-plasma interactions. In 1972 we reported that dilute plasma obtained from a diabetic patient who died because of disseminated intravascular coagulation would potentiate ADP-induced platelet aggregation in vitro.[22] Subsequently, we found that such activity could be found in diabetic patients without apparent vascular disease,[23–25] and even in patients with impaired glucose tolerance. Some laboratories have not been successful in finding such activity[26–28] while others have.[29,30] As cited above, some investigators have demonstrated spontaneous aggregation of platelets from diabetics when platelets are mixed in vitro with platelet-rich plasma, suggesting that plasma-platelet interactions may be operative in the diabetic state.

As reported previously,[31,32] we have performed studies to partially define the nature of the platelet-active plasma factor(s). One of these has been termed platelet aggregation enhancing factor (PAEF), and represents the potentiation by plasma from a diabetic of platelet aggregation produced by subthreshold amounts of ADP. Active peaks have been found by Sephadex G-100 and G-50 separation, and the activity can be blocked by prostacyclin and by aspirin treatment of the normal platelet donor. Work is underway to further characterize the active peaks.

We have also explored the possibility that immune complexes may enhance platelet activity in diabetes mellitus. Using five different screening techniques, we have reported that increased immune complex activity could be found in 25–29% of sera from 148 heterogeneous diabetic patients.[33,34] Immune complex scores correlated positively with sensitive indices of renal involvement. While PAEF and platelet aggregation were not studied concurrently, many patients had previously displayed increased platelet aggregation and/or PAEF activity in our laboratories. We therefore set out to determine if purified immune complexes from diabetics were

TABLE 3
Experimental diabetes: platelet function

Author	Year	Agent*	Species	Results
Honour[15]	1977	Al	Rabbit	↑ ADP aggregation (in vivo)
Kuwashima[16]	1979	Al	Rabbit	↑ collagen aggregation, adhesion
Eldor[17]	1978	STZ	Rat	↑ thrombin, ADP aggregation†
Johnson[18]	1979	STZ	Rat	↑ ADP, collagen aggregation
Gerrard[19]	1980	STZ	Rat	↑ thrombin stimulated arachidonic acid release†
Murray[20]	1980	Al	Rabbit	↑ ADP aggregation
Rosenblum[21]	1981	—	Mouse (ob/ob, db^+/db^+)	↑ aggregation time – cerebral + mesenteric microvessels

*Al = alloxan, STZ = streptozotocin; †washed platelets.

platelet active.[35] When purified immune complexes from diabetics were added to platelets stimulated with low concentrations of ADP, a second phase of platelet aggregation and the platelet release reaction was produced. Soluble immune complexes, therefore, appear to be the first well-characterized platelet aggregating factors from the sera of diabetic patients.

Platelet arachidonic acid metabolism. There are seven studies in which platelet arachidonic acid metabolism has been measured in IDDM subjects who are free from vascular disease.[5–7,36–39] These are summarized in Table 4. In 1977 we reported that platelets from IDDM would release increased amounts of immunoreactive PGE_2 when stimulated by ADP, epinephrine, collagen, or arachidonic acid. The majority of these patients were free from diabetic angiopathy. Ziboh showed that there was increased thromboxane synthesis by platelets from diabetics, and that those with vascular disease had the greatest TXA_2 release.[36] Ziboh[36] and Stuart[7] showed that platelets from pregnant diabetic mothers free from vascular disease synthesized increased amounts of TXA_2 or malonyldialdehyde, as indices of increased arachidonic acid metabolism. Chase[37] showed that serum from IDDM children who were presumably free from vascular disease had increased levels of PGE_2 and $PGF_{2\alpha}$. In recent studies, Butkus[38] and Halushka[6] have shown increased thromboxane synthesis by platelets from diabetics, some of whom had angiopathy. Similar results have been found by Subbiah[39] in diabetes occurring spontaneously in the BB-Wistar rat.

It appears that increased platelet metabolism of arachidonic acid may occur in IDDM patients before the appearance of clinically apparent vascular disease and in experimental diabetes. When vascular disease is present in diabetic patients, increased platelet arachidonic acid metabolism may also be seen.

Beta-thromboglobulin (β-TG). Beta-thromboglobulin is a platelet-specific protein that is released by the platelet alpha granules after stimulation by thrombin, ADP, or collagen. Plasma β-TG levels may reflect in vivo platelet aggregation and release. Results from seven studies of β-TG in diabetes are given in Table 5.[9,13,40–45] In the majority of these studies, elevated plasma β-TG levels have been found in groups of patients who were predominantly free from vascular disease. In particular, Preston[40] and Burrows[42,43] have shown that newly diagnosed IDDM who are free from angiopathy may have elevated plasma β-TG levels. These findings indicate that in vivo platelet activation and release may occur before the appearance of clinically significant diabetic vascular disease.

Platelet survival. Studies on platelet survival in diabetes have also shown that there may be an increased turnover of platelets in vivo in animals and in man[18,46–51] (Table 6). Reduced platelet survival is often seen in diabetics with and without vascular disease. Increased platelet regeneration after aspirin in IDDM patients without retinopathy has been reported.[49,51] Measured by this technique, platelet life span was shortened in groups of IDDM with and without retinopathy when compared with controls. Studies of decreased platelet survival in rats[18] are in accord with these studies in uncomplicated human IDDM. Collectively, these data support the view that increased platelet turnover occurs in vivo in uncomplicated IDDM.

Metabolic control. It is still not clear whether careful metabolic control will restore platelet function to normal in diabetic patients[10,14,40,52,53] (Table 7). Beneficial effects of insulin therapy on platelet function include a fall in β-TG levels re-

TABLE 4
Increased platelet prostaglandins in insulin-dependent diabetes mellitus

Author	Year	Findings
Halushka[5]	1977	↑ PGE_2: ± angiopathy
Ziboh[36]	1979	↑ TXA_2 synthesis: vascular > nonvascular > NL TXA_2 synthesis: pregnant DM
Stuart[7]	1979	↑ MDA: pregnant DM, no angiopathy
Chase[37]	1979	↑ PGE_2, $PGF_{2\alpha}$: children, ? angiopathy
Butkus[38]	1980	↑ TXB_2: with angiopathy only
Subbiah[39]	1980	↑ TXB_2: BB Wistar rat
Halushka[6]	1981	↑ TXB_2: ± angiopathy

TABLE 5
Elevated beta-thromboglobulin in diabetic patients

Author	Year	Comments
Preston[40]	1978	Newly diagnosed diabetics
Borsey[41]	1980	No microangiopathy
Burrows[42,43]	1978, 1981	Newly diagnosed diabetes, no microangiopathy
Paulsen[9]	1981	32%—retinopathy
Schernthaner[44]	1981	No microangiopathy*
Janka[45]	1981	No microangiopathy*
Breddin[13]	1981	Some with angiopathy

*Questionable significance or NS.

TABLE 6
Platelet survival in diabetic patients

Author	Year	Vasc. Dis.	Findings
Abrahamsen[46]	1968	+	Reduced
Ferguson[47]	1975	0	Reduced
Dassin[48]	1978	+, 0	Reduced in 33%
Johnson[18]	1979	0	Reduced (rats)
Paton[49]	1979	0	↑Regeneration time (ASA)
Jones[50]	1981	+, 0	Reduced (+), Normal (0)*
Tindall[51]	1981	+, 0	↑Regeneration time (ASA)

*No effect of control with insulin.

TABLE 7
Metabolic control and platelet function

Author	Year	Findings
Peterson[52]	1977	Prolonged epinephrine lag phase
Hilsted[53]	1980	Increased ADP aggregation after hypoglycemia, then normal
Preston[40]	1978	Fall in β-TG levels
Janka[10]	1981	Insulin Rx caused increased ADP, epinephrine, and collagen aggregation
Juhan[14]	1982	Insulin Rx caused decreased whole blood aggregation (RBC?)

ported by Preston[40] and a decrease in whole blood aggregability shown by Juhan.[14] A prolongation of the lag phase that occurs before second-wave platelet aggregation has been shown when very high concentrations of epinephrine were used in vitro.[15] The physiologic significance of this latter finding may be questioned, however, since the platelet release reaction was unaffected. Several observers have reported increased sensitivity to platelet aggregating agents in vitro after insulin.[10,53]

ENDOTHELIAL FUNCTION IN INSULIN-DEPENDENT DIABETES MELLITUS

Prostacyclin (PGI_2). Prostacyclin is a product of endothelial cell arachidonic acid metabolism; it is antiplatelet-aggregatory and vasodilatory. It has been suggested that this material may protect the endothelium against platelet deposition. Many studies have measured endothelial cell PGI_2 synthesis in the diabetic state to explore the idea that diminished production could interact with "sensitized" platelets to support platelet deposition on endothelium. Eleven studies from six different laboratories are summarized[19,39,54–62] (Table 8). There is remarkable agreement, considering the different species studied and the heterogeneity of the diabetic state. All studies have shown decreased PGI_2 production from arteries or veins taken from diabetic patients or diabetic animals. In several studies, levels of the stable prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$, were found to be low. In three studies, PGI_2 release could be returned to normal by insulin therapy or by pancreatic islet transplantation.

Studies with rats support the view that decreased endothelial cell production of PGI_2 occurs in the diabetic state before vascular disease develops. While this may also be true in man, it has not been clear from the studies to date whether nondiseased small or large arterial vessels from IDDM have decreased PGI_2 release. The results to date support the concept that platelet-endothelial interactions may promote thrombosis in the diabetic state.

Von Willebrand factor (VIII R:WF). Plasma levels of this endothelial-derived protein may be elevated in diabetes. Studies of von Willebrand factor in diabetes have been summarized.[1,63] It is likely its elevated plasma level in diabetes reflects endothelial damage. Since it is a factor important for platelet adhesion, VIII R:WF may contribute in some way to the platelet abnormalities already described. However, while very low levels of VIII R:WF are associated with a bleeding tendency, it is not clear that high levels will promote platelet dysfunction and/or thrombosis.

Fibrinolysis. An important function of the intact endothelium is the production of plasminogen activator, an enzyme of the fibrinolytic system responsible for the conversion of plasminogen to plasmin. Plasmin, by degrading fibrin, acts as a limiting factor in the growth of thrombi. Almer has reported that vessel wall plasminogen activator activity is low in diabetes.[64,65] Plasma fibrinolytic activity may be normal,[66,67] decreased,[68] or increased.[69,70] While decreased release of plasminogen activator together with reduced plasma fibrinolytic activity might promote thrombosis, further investigation is needed in this area of research in diabetes mellitus to resolve these conflicting reports.

PLATELET MICROTHROMBI IN EXPERIMENTAL DIABETES

One of the arguments challenging the hypothesis that platelets contribute to microvascular disease has been the paucity of histopathologic evidence that platelets can be found in microvascular lesions. While there are scattered reports of platelet microthrombi in diabetic microangiopathy,[71] there had not been a suitable animal model until recently. Ishibashi and co-workers have now reported that microthrombi can be seen in retinal microvessels after 9–12 mo of experimental diabetes in rats.[72] The thrombi appear to consist primarily of

TABLE 8
Prostacyclin production in diabetic patients

Author	Year	Species	Vessels	Findings
Harrison[54,55]	1978, 1980	Rat	Aorta, renal cortex	Decreased*
Johnson[56,57]	1979, 1981	Man	Arteries at surgery	Decreased
Silberbauer[58–60]	1979, 1981	Man, rat, pig	Vein	Decreased
Subbiah[39]	1980	Rat	Aorta	Decreased
Carreras[61]	1980	Rat	Aorta	Decreased
Gerrard[19]	1980	Rat	Aorta	↓ 6K $PGF_{1\alpha}$*
Dollery[62]	1979	Man	Plasma	↓ 6K $PGF_{1\alpha}$

*Reversed with insulin therapy.

TABLE 9
Platelet and endothelial function in insulin-dependent diabetes mellitus

Abnormal platelet function
↑ platelet aggregation
PAEF and immune complex interaction
↑ PGs and metabolites
↑ β-TG
↓ survival
Abnormal endothelial function
PGI_2 (↑ with control)
↑ VIII R:WF
↓ plasminogen activator
Experimental DM → microthrombi
Vascular disease slowed by antiplatelet drugs (?)

platelets and fibrin. These studies support the hypothesis that platelet dysfunction may contribute to microocclusive vascular disease in diabetes.

ANTIPLATELET AGENTS IN DIABETIC VASCULAR DISEASE

There are three ongoing studies in which antiplatelet drugs are being used to determine if the rate of progression of diabetic vascular disease can be modified. The first of these is a V.A. Cooperative Study, which started in 1977 and will be completed in 1983. Adult male diabetic patients who have had recent amputations for gangrene have been randomly assigned to aspirin (325 mg t.i.d.) plus dipyridamole (75 mg t.i.d.) or placebo. Primary endpoints of vascular death, myocardial infarction, and subsequent amputation for gangrene are closely monitored, as are secondary endpoints such as retinopathy, angina, and claudication.

Two studies are using antiplatelet agents in an attempt to modify the progression of diabetic retinopathy. One of these is a European study and the second is an NIH-sponsored study, the Early Treatment for Diabetic Retinopathy Study (ETDRS). Results from these studies will provide important information bearing on the role of altered platelet function in diabetic vascular disease.

DISCUSSION

Findings of platelet and endothelial function in IDDM without vascular disease are summarized in Table 9. It is clear that many abnormalities of platelet and endothelial function that could promote thrombosis are present in IDDM before apparent vascular disease. Furthermore, experimental models have been developed, and there is evidence that PGI_2 release (and, perhaps, platelet function) may be normalized by tight metabolic control with insulin. Additional evidence in support of the platelet-endothelial hypothesis may be provided by ongoing antiplatelet studies in diabetic patients.

It should be recognized, however, that much remains to be done. A great number of unanswered questions persist. Among these are:

1. What is the true sequence of events in diabetic vascular disease? Does endothelial damage precede or follow platelet activation in diabetes?
2. Does altered lipid or lipoprotein metabolism in diabetes relate to altered platelet and/or endothelial function?
3. Does protein glycosylation, a predictable consequence of the uncontrolled diabetic state, play a role in platelet or endothelial abnormalities?
4. Is platelet adhesion, as distinct from aggregation, altered in the diabetic state?
5. What is the effect of careful long-term control with diet, oral agents, and/or insulin on platelet and/or endothelial abnormalities seen in diabetes?
6. Do platelets from diabetics release platelet growth factors that differ qualitatively or quantitatively from those released by platelets from nondiabetic subjects?
7. Do platelet and/or endothelial defects have any relation to either large or small vessel disease in diabetes mellitus?

Many questions remain unanswered and many more could be posed. It is hoped that research will pursue attempts to answer some or all of these questions. Until then, the platelet-endothelial theory remains only an attractive hypothesis as it may relate to the genesis of diabetic microvascular disease.

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