Platelet Coagulant Activities in Arterial Occlusive Disease of the Eye

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SUMMARY Ischemic optic neuropathy and retinal arterial occlusion are 2 forms of arterial occlusive disease affecting the eye. Reports in the literature suggest platelet hyperactivity in acute arterial occlusive diseases affecting other organ systems. Therefore, 14 patients with ischemic optic neuropathy and 17 patients with central or branch retinal artery occlusion were studied to determine whether platelets have a role in the pathogenesis of these vascular occlusive disorders. The results of the following investigations were no different in these patients compared with those in 18 control patients with non-vascular eye diseases: prothrombin times, partial thromboplastin times, plasma fibrinogen, factor V, factor VIII, platelet counts and threshold concentrations of ADP, epinephrine and collagen resulting in secondary platelet aggregation and serotonin release. In contrast, platelet coagulant activities concerned with the early stages of intrinsic coagulation were significantly increased in patients with retinal artery occlusion without hypertension or type IV hyperlipoproteinemia, but generally normal in patients with ischemic optic neuropathy and in patients with retinal artery occlusion associated with hypertension, type IV hyperlipoproteinemia, diabetes mellitus and generalized atherosclerosis. These results are consistent with a platelet contribution to retinal arterial occlusive disease in patients without other known contributing factors such as hypertension, serum lipid abnormalities, diabetes mellitus and generalized atherosclerosis and may have implications regarding prophylaxis.

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It has been shown that platelets participate in the initiation of intrinsic coagulation and promote subsequent intrinsic coagulation reactions that result in thrombin generation. Platelet coagulant activities concerned with the initiation and early stages of intrinsic coagulation were previously found to be increased 2-4-fold in patients with transient cerebral ischemic attacks and normal serum lipids but not in patients with transient ischemic attacks associated with type IV hyperlipoproteinemia. Similar abnormalities of platelet coagulant activities were also found in patients with acute primary retinal vein thrombosis, and normal results were found in patients with retinal vein occlusion secondary to other diseases. These observations suggest that platelets contribute to the development of acute arterial and venous occlusive disease in certain patients, but other factors such as hypertension, atherosclerosis, serum lipid abnormalities and diabetes mellitus may be more important pathogenetic factors in other patients. To examine the possible role of platelets in the pathogenesis of arterial occlusive diseases of the eye, we have studied platelet coagulant activities, platelet...
aggregation and secretion, plasma coagulation and serum lipids in 14 patients with ischemic optic neuropathy and 17 patients with retinal artery occlusion. We have chosen the eye as a model of arterial occlusive disease because of the availability of specific diagnostic methods and because the limited extent of acute infarction minimizes the likelihood that abnormal results are secondary to extensive tissue damage or inflammation.

Materials and Methods

Fourteen patients with ischemic optic neuropathy and 17 patients with arterial occlusive disease of the retina (13 with branch, 4 with central retinal artery occlusion) were studied. They ranged in age from 25 to 81. (The characteristics of these patients are shown in table 1). The ophthalmoscopic criteria for diagnosis of ischemic optic neuropathy included a pale swollen disc with narrowed arterioles at the disc and a normal appearance of the retina unless infarction was present in the area of the cilioretinal artery; in addition venous tortuosity and hemorrhage at the disc in the nerve fiber layer may have been present. Patients with ischemic optic neuropathy were clearly distinguished from those with retinal artery occlusion. The diagnostic criteria for this distinction included characteristic edema of the entire retina (central retinal artery occlusion) or a segment of the retina (branch occlusion) with evidence of interruption of blood flow in retinal arteries (e.g., "boxcarring") and a normal appearance of the optic disc. The diagnosis was established in all patients by detailed ophthalmic evaluation including indirect ophthalmoscopy. Stereoscopic color fundus photography and Goldmann perimetry were performed in all patients. Fluorescein angiography was carried out when necessary to confirm the diagnosis in all patients with retinal artery occlusion. All patients were evaluated medically by one of us (PNW). The erythrocyte sedimentation rate (Westergren) was determined as an emergency, and if abnormal, temporal artery biopsy was performed to establish a diagnosis of giant cell arteritis. Patients with giant cell arteritis were excluded from the study. Laboratory investigations included complete blood count, urinalysis, serum lipids, glucose tolerance test, blood chemistries and electrocardiogram. Patients and normal controls were studied after a 14 hour period of fasting, and they denied having received medications known to affect platelets or serum lipids for at least 2 weeks. Thirty-eight normal subjects were studied to establish normal values. Eighteen patients with non-vascular, non-thrombotic eye disease (9 males, 9 females), age range 26–70 (mean 57) also were studied, none of whom had detectable atherosclerotic or thrombotic vascular disease elsewhere or hypertension, diabetes mellitus or serum lipid abnormalities. Platelet studies were performed 10 days to 15 months after the initial manifestation of the arterial occlusion.

Preparative Procedures

Nine volumes of blood were collected by clean venipuncture directly into 1 vol of 3.8% trisodium citrate with plastic containers and equipment. Platelet-rich plasma and high spun platelet-poor plasma were prepared as previously described. Platelets were washed and suspensions were stored as previously described. Platelets were counted by phase contrast microscopy and electronically with a Model ZBI Particle Counter (Coulter Electronics, Hialeah, FL).

Coagulation Assays

Determinations of one-stage prothrombin times and activated partial thromboplastin times and assays for fibrin degradation products, fibrinogen and factors V and VIII, were done as previously described. Platelet aggregation and serotonin release studies were done as previously described with ADP, epinephrine and collagen. Threshold concentrations of each agent resulting in secondary aggregation and release of more than 20% of [14C] 5-HT were determined.

Platelet Coagulant Activities

The platelet coagulant activities assayed include: 1) Contact-product-forming activity, the capacity of normal platelets to respond to ADP and participate in the activation of Factor XII; 2) collagen-induced coagulant activity, the capacity of collagen-stimulated platelets to participate in the initiation of intrinsic coagulation by an alternative mechanism in the ap-

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parent absence of Factor XII,\textsuperscript{23, 24} 3) intrinsic factor-X\textsubscript{a}-forming activity, by which platelet membrane components become available and promote the interactions of factors X\textsubscript{a}, VIII and X to activate factor X in the presence of calcium;\textsuperscript{26, 27} and 4) platelet factor 3 activity by which platelet membrane phospholipoproteins become available and promote the interactions of factors X\textsubscript{a} and V to activate prothrombin in the presence of calcium.\textsuperscript{28, 29} These platelet coagulant activities were assayed by modifications\textsuperscript{30} of previously described methods and were expressed as percentages of normal platelets by reference to a double logarithmic plot of clotting time and platelet concentration.\textsuperscript{29}

**Diagnosis of Hyperlipoproteinemia**

The diagnosis of type IV hyperlipoproteinemia was based on determinations of serum cholesterol, triglycerides and lipoprotein electrophoresis after a fast of more than 14 h.\textsuperscript{30}

**Statistical Methods**

Results were expressed as mean and standard error of the mean (SEM) and groups were compared by Student's $t$-test.

**Results**

Patients with ischemic optic neuropathy were studied between 10 days and 15 months after visual loss, and those with retinal artery occlusion were studied between 2 days and 6 months after visual loss (Table 1). Patients with retinal artery occlusion were retrospectively classified into 2 groups on the basis of the results of serum lipid determinations and presence or absence of hypertension. Group A consisted of 11 patients with neither hypertension nor abnormalities of serum lipids, in whom studies were carried out on average 1.6 months after visual loss. Group B consisted of 6 patients all of whom had hypertension and/or type IV hyperlipoproteinemia, in whom the average time of study after visual loss was .10 month.

**Coagulation Studies Results (Table 2)**

The results of determinations of prothrombin time, partial thromboplastin time and concentrations of factors V, VIII and fibrinogen for patients with ischemic optic neuropathy and retinal artery occlusion were compared with those for control patients. No significant differences were observed. When results were analyzed separately for group A and B patients with retinal artery occlusion, no significant differences were observed.

**Platelet Aggregation, Serotonin Release and Platelet Counts (Table 3)**

The mean threshold concentrations of ADP, epinephrine and collagen resulting in secondary aggregation and the release of $\geq 10\%$ $[^{14}C]$ 5-HT for patients with ischemic optic neuropathy and retinal artery occlusion were compared with results for control patients and normal subjects. No significant differences were observed. Platelet counts were also similar in control patients and in those with ischemic optic neuropathy and retinal artery occlusion. When the results for group A and B patients with retinal artery occlusion were analyzed separately, no significant differences were observed.

**Platelet Coagulant Activities (Table 4)**

Platelet coagulant activities concerned with the initiation and early stages of intrinsic coagulation (contact forming product activity, collagen-induced coagulant activity and intrinsic factor-X\textsubscript{a}-forming activity) were significantly increased in patients with retinal artery occlusion compared with results from control patients and normal subjects. The results of assays for platelet factor 3 activity after incubation of platelets with either collagen or kaolin in patients with retinal artery occlusion were no different from the results in control patients with nonvascular eye disease. When the results of platelet coagulant activity assays in patients with retinal artery occlusion were analyzed separately for group A patients and group B patients, normal results were found consistently in those with hypertension and/or type IV hyperlipoproteinemia (group B). In contrast, patients with neither hypertension nor serum lipid abnormalities (group A) had significantly increased results of assays for contact forming product activity, collagen-induced coagulant activity and intrinsic factor-X\textsubscript{a}-forming activity. Patients with ischemic optic neuropathy had results for platelet coagulant activities not significantly different from those in patients with nonvascular eye diseases.

**Discussion**

Retinal ischemia may result from circulatory obstruction proximal to the eye (e.g. the carotid...
artery) or it may be due to occlusion of the retinal arteries themselves in a variety of disorders including systemic vascular disease, hypertension, diabetes mellitus, giant cell arteritis, the collagen diseases, and sickle cell hemoglobinopathies.\textsuperscript{31} Retinal artery occlusion may also be caused by platelet-fibrin emboli or emboli of atheromatous cholesterol-containing material.\textsuperscript{31-32} In a large group of patients, however, the cause of retinal arterial occlusion remains obscure.

Platelets function in hemostasis by adhering to subendothelial surfaces exposed after vascular injury, aggregating to form a platelet thrombus and secreting the contents of their granules into the surrounding plasma. Platelets have also been shown to participate in the initial reactions of intrinsic coagulation and to promote subsequent coagulation factor interactions resulting in the formation of thrombin.\textsuperscript{12} In addition, the central role of platelets in the formation of arterial thrombi and in the initiation of vascular injury has been stressed.\textsuperscript{33} Enhanced sensitivity of platelets to aggregating agents,\textsuperscript{34-38} a tendency for platelets to aggregate spontaneously,\textsuperscript{37} and evidence for the presence of circulating platelet aggregates in blood\textsuperscript{39} have been demonstrated in patients with cerebral vascular disease and in acute arterial occlusive disease affecting other organ systems. Previously we demonstrated an association between platelet coagulant hyperactivity and transient cerebral ischemic attacks in a group of patients with normal serum lipids.\textsuperscript{39} These findings suggest that platelets are involved in the pathogenesis of acute arterial occlusive disease involving the cerebral circulation.

Our present study of patients with retinal artery occlusion was done to investigate the possibility that platelets are also involved in the pathogenesis of acute arterial occlusive disease of the eye. Seventeen patients with retinal artery occlusion were divided into 2 categories. Group A consisted of 11 patients without either hypertension or serum lipid abnormalities. These patients had significant elevations of platelet coagulant activities concerned with the initiation and early stages of intrinsic coagulation. Group B included 6 patients with type IV hyperlipoproteinemia and/or hypertension and with a higher incidence of diabetes mellitus and vascular occlusive disease involving cerebral, peripheral and coronary circulations. Platelet coagulant activity assays were normal in group B patients. There was no evidence for abnormalities of blood coagulation, platelet aggregation responses to ADP, epinephrine or collagen or platelet secretion in any of the patients with retinal artery occlusion. These results suggest that retinal arterial occlusive or embolic disease can arise from diverse mechanisms. Platelets would appear to be involved, possibly by formation of platelet-fibrin emboli, in patients without hypertension or hyperlipidemia (group A), whereas retinal artery occlusion appears to be related to other factors, such as hypertension, hyperlipidemia and generalized atherosclerosis, in other patients (group B), in whom platelets are normal. It seems unlikely that platelet abnormalities occur as a consequence of retinal artery occlusion since abnormal results were not detected in group B.

It is interesting to consider the present results in the light of our previous studies of patients with transient cerebral ischemic attacks\textsuperscript{13} and other patients with

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\textbf{Determination} & \textbf{Ischemic optic neuropathy} & \textbf{Retinal artery occlusive} & \textbf{Control patients} & \textbf{Normal values} \\
 & \textbf{(n = 14)} & \textbf{(n = 17)} & \textbf{(n = 18)} & \textbf{(n = 38)} \\
\hline
ADP (\textmu M) & 3.0 ± 0.8 & 2.1 ± 0.5 & 2.4 ± 0.4 & 3.0 ± 0.2 \\
Epinephrine (\textmu M) & 1.0 ± 0.5 & 1.7 ± 0.5 & 1.4 ± 0.3 & 2.7 ± 0.3 \\
Collagen (g/l) & 1.6 ± 0.5 & 0.9 ± 0.3 & 0.7 ± 0.1 & 1.2 ± 0.1 \\
Platelet count (\texttimes 10^9/l) & 263 ± 23 & 231 ± 12 & 226 ± 12 & 150-300 \\
\hline
\end{tabular}
\caption{Platelet Aggregation, Serotonin Release and Platelet Counts}
\end{table}

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\textbf{Contact product forming activity} & \textbf{Collagen-induced coagulant activity} & \textbf{Intrinsic factor-Xa forming activity} & \textbf{Platelet factor 3 activity} \\
\hline
\textbf{Retinal artery occlusion (17)*} & 138 ± 16† & 193 ± 28† & 159 ± 24† & 115 ± 10 \\
\textbf{Group A (11)} & 169 ± 18† & 251 ± 34† & 205 ± 29† & 120 ± 15 \\
\textbf{Group B (6)} & 93 ± 16 & 106 ± 15 & 92 ± 22 & 94 ± 9 \\
\textbf{Ischemic optic neuropathy (14)} & 112 ± 21 & 122 ± 15 & 119 ± 24 & 113 ± 9 \\
\textbf{Non-vascular eye diseases (18)} & 98 ± 9 & 85 ± 8 & 91 ± 7 & 105 ± 9 \\
\textbf{Normal range} & 42-158 & 62-154 & 30-170 & 40-160 \\
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\end{tabular}
\caption{Platelet Coagulant Activities}
\end{table}

Data shown represent mean ± SEM. *Figures in parentheses denote number of patients in group. †p < 0.001 when value compared with result in patients with non-vascular eye disease. The rest of the values in patients with retinal artery occlusion and ischemic optic neuropathy were not significantly different (p <0.05) from corresponding results in patients with non-vascular eye disease.
retinal vein thrombosis. Patients with transient ischemic attacks and normal serum lipids were found to have 2 to 4-fold elevation in platelet coagulant activities concerned with the early stages of intrinsic coagulation, whereas patients with transient ischemic attacks and type IV hyperlipoproteinemia had normal platelet studies. Patients with acute primary retinal vein occlusion had similar platelet abnormalities, whereas those with retinal vein occlusion secondary to other conditions, such as hypertension, diabetes mellitus, type IV hyperlipoproteinemia and associated vascular occlusive disease, had normal platelet studies. In all 3 studies there was a tendency for patients with vascular occlusive disease associated with platelet abnormalities to be younger than those with normal platelets and a high incidence of hypertension, diabetes mellitus, type IV hyperlipoproteinemia and atherosclerosis. It can be inferred from these observations that platelets have a role in the pathogenesis of arterial occlusive disease of the eye and brain and venous occlusive disease of the eye in certain patients when local conditions prevail whereas in other patients pathogenetic factors such as hypertension and atherosclerosis predominate and platelets are normal. It is also evident that the platelet coagulant hyperactivity observed in patients with diverse thrombotic diseases is not specifically associated with a single type or location of thrombotic disease. Thus factors other than platelets must determine the type and location of blood vessels involved.

Our findings in patients with retinal artery occlusion are consistent with clinical, pathological and experimental evidence that many episodes of transient occlusion of retinal arteries arise from embolization of material consisting either of platelet-fibrin thrombi or atheromatous material, often found in association with ulcerative atheromatous plaques in the carotid arteries. These observations and our findings of hyperactive platelets in some patients with retinal artery occlusion are supported by reports of increased platelet adhesiveness and an increased percentage of both spontaneously and ADP-aggregated platelets in patients with retinal arterial occlusion.

The clinical characteristics and natural history of idiopathic ischemic optic neuropathy have been carefully examined, but the pathogenesis remains unknown. The syndrome generally occurs in patients in the sixth decade or older, about half of whom have mild hypertension, but there is no demonstrated association with extracranial carotid occlusive disease and no increased incidence of stroke. Our findings in patients with ischemic optic neuropathy do not contribute to an understanding of its pathogenesis. It therefore seems reasonable to regard our patients with ischemic optic neuropathy as an additional group of control patients without evidence of thrombotic eye disease to which patients with retinal artery occlusion may be compared. Our results are consistent with those of Kok and Watson who found that both spontaneous and ADP-induced platelet aggregation were normal in patients with ischemic optic neuropathy and abnormal in patients with retinal artery occlusion.

Our results in patients with retinal artery occlusion suggest 2 alternative approaches to patient management. In group A patients with platelet coagulant hyperactivity without associated hypertension, hyperlipidemia or evidence of generalized atherosclerosis it would seem rational to evaluate drugs that inhibit platelet function in preventing recurrences of vascular occlusive disease. In group B patients with normal platelet coagulant activities, attempts should be made with diet, drugs and other measures to control associated diseases such as hypertension, hyperlipidemia, diabetes mellitus and resultant atherosclerosis.

The mechanism by which platelet coagulant hyperactivity develops in group A patients is unknown. Platelets might be activated by exposure to ulcerated atheromatous plaques with resultant platelet coagulant hyperactivity and embolization of platelet-fibrin masses. Alternatively, in patients without underlying vascular disease platelet coagulant hyperactivity could result from undefined intravascular stimuli or hyperactive platelets could be released from the bone marrow. It has been suggested that in some patients with arterial occlusive disease, platelet turnover is increased and more metabolically and functionally active platelets appear in the circulation. However, it is not known whether similar mechanisms exist in patients with retinal arterial occlusive disease.

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