Platelet Survival and the Development of Coronary Artery Disease in the Young Adult: Effects of Cigarette Smoking, Strong Family History and Medical Therapy

Valentin Fuster, M.D., James H. Chesebro, M.D., Robert L. Frye, M.D., and Lila R. Elveback, Ph.D.

with the technical assistance of James M. Byrne

SUMMARY  Cigarette smoking or a strong family history of coronary disease was present in 46 of 50 symptomatic patients with coronary artery disease who were younger than 50 years of age. We recorded a shortened platelet survival half-life (< 92 hours) with 51Cr in 60% of these patients, in 56% of apparently normal persons of the same age who smoked or had a strong family history of coronary disease, and in only 14% of normal persons who did not smoke and had no family history (p < 0.01). Lengthening of the shortened platelet survival toward normal occurred in coronary patients given dipyridamole plus aspirin and in apparently normal smokers who discontinued smoking (p < 0.01).

The study suggests a positive relationship among cigarette smoking, strong family history of coronary disease and platelet activation in the process of coronary atherosclerosis in the young adult.

ALTHOUGH there is convincing evidence that platelets are important in the thrombotic and embolic complications of atherosclerosis, an important role in atherogenesis has only recently been proposed. Both animal and cell culture studies indicate that platelets, by adhering to subendothelial structures in areas of endothelial cell desquamation and releasing a platelet mitogenic factor, stimulate the intimal smooth muscle cell proliferation of the early atherosclerotic lesion. Furthermore, there is evidence that collagen organization of platelet-fibrin thrombi can also contribute to the growth and progression of atherosclerotic plaques in animals and humans.

Platelet survival time has been found to be short in patients with coronary atherosclerotic disease and tends to normalize with the administration of platelet inhibitor drugs. We studied young adults with coronary artery disease to determine the frequency of occurrence of shortened platelet survival in this group of patients and to assess the response to platelet-inhibitor therapy. Because of evidence that cigarette smoking and strong family history are high-risk factors for the development of coronary atherosclerotic disease in the young, platelet survival was estimated in asymptomatic young adults with these risk factors. In cigarette smokers, the effect of discontinuation of smoking on platelet survival was also evaluated.

Methods

Clinical Groups

We studied three groups of subjects, ages 50 years or younger, who agreed to participate in the study. Group 1 consisted of 50 patients (43 males and seven females, ages 34–48 years, average age 41 years) who had symptomatic coronary artery disease and who, at arteriography, had at least one coronary artery narrowed 50% or more in luminal diameter. The severity and extent of the disease were evaluated by dividing the coronary artery tree into 27 segments and coding each segment with any abnormality. The numbers of segments with complete occlusion, 70% or more stenosis, 50% or more stenosis and 10% or more stenosis were independently summed and studied in relation to the platelet survival measurements. No patient had clinical evidence of atherosclerotic disease in vessels other than the coronary arteries, and none had undergone surgical coronary revascularization. The first symptom of coronary artery disease was angina pectoris in 36 of these patients and myocardial infarction in the other 14.

Group 2 consisted of 25 subjects (17 males and eight females, ages 29–47 years, average 38 years) who were apparently healthy but who had risk factors for coronary artery disease as defined below.

Group 3 included 28 subjects (13 males and 15 females, ages 29–49 years, average 39 years) who were apparently healthy and who had none of the risk factors for coronary artery disease defined below.

Risk Factors

Cigarette smoking. The number of pack-years of smoking was determined from the patient by at least two physicians. A positive smoking history was considered to be 10 pack-years or more. In group 1, 40 of
50 patients were cigarette smokers, although only 14 were currently smoking. In group 2, 22 of 25 subjects were cigarette smokers, and all of them were currently smoking.

Strong family history — that is, first-degree relatives with coronary artery disease at age 50 years or younger. This was first determined by an interview by at least two physicians and then was further evaluated by a telephone interview with each patient and/or first-degree relatives. Strong family history was present in 17 of 50 patients in group 1 and in three of 25 in group 2.

Serum cholesterol and triglyceride levels. These were documented and analyzed as previously described. Although 11 patients of group 1 had serum cholesterol or triglyceride values at or above the ninety-fifth percentile for a patient’s age and sex, in only three patients were these values above 300 mg/dl for cholesterol or 250 mg/dl for triglycerides.

Systemic hypertension, defined as blood pressure of 160/95 mm Hg or more on three occasions.

Diabetes mellitus, defined as two fasting plasma glucose values of 120 mg/dl or more or insulin dependence. Only one patient in group 1 had both systemic hypertension and diabetes.

Platelet Survival Method

In all patients with symptomatic coronary disease, platelet survival time was measured within 3 months after coronary arteriography. All patients had normal platelet counts, and platelets were labeled with $^{51}$Cr in a closed system as follows. In the blood bank, 425 ml of blood was collected in a Fenwal Double Plasma- pheresis Double Blood-Pack (the bag, no. 1 and its satellite bag were discarded) with the large collecting bag (no. 2) containing 75 ml of acid-citrate-dextrose (ACD, USP formula A). After collection, the bag containing the blood was removed for processing; the “J” valve portion of the tubing was kept in place, and the patency was maintained by a slow saline drip. Platelet-rich plasma (PRP) was prepared by centrifugation at 300 $\times$ g for 12 minutes at room temperature and then was put in the satellite transfer bag with a plasma extractor. The bag containing the red cells was removed, and the cells were transfused into the patient. Then, the patient was allowed to leave the blood bank for 2 hours, the time necessary for completion of the platelet labeling procedure. After the PRP was separated, its pH was adjusted to 6.5 with sterile 0.15 M citric acid (0.1 ml/10 ml plasma). Platelet-poor plasma (PPP) and a platelet pellet were prepared by centrifugation of PRP at 1500 $\times$ g for 15 minutes. After centrifugation, two 20-ml portions of the PPP were drawn into syringes and saved, and then all but 10 ml of the remaining PPP was removed into a 300 ml Fenwal transfer pack. The platelets were gently resuspended into the 10-ml residual PPP. (The procedure required about 15 minutes, and the bag was not directly kneaded.) This platelet suspension was then incubated with about 300 $\mu$Ci $[^{51}$Cr] NaCrO$_4$ (New England Nuclear Co.) for 20 minutes at room temperature. Most of the nonradioactive PPP and 200 ml of sterile air were then added, and the pellet was re-formed by centrifuging at 1500 $\times$ g for 15 minutes. The residual radioactive PPP was completely decanted by gravity flow, with meticulous care taken to avoid disturbing the pellet. Nonradioactive PPP (20 ml) was carefully layered over the platelet pellet and then discarded by decantation. This maneuver removes any residual radioactivity in the bag. The presence of air in the bag facilitates this process by maintaining the overall configuration of the bag during decantation. This labeled platelet pellet was gently suspended in 20 ml of nonradioactive PPP. Contaminating red cells were removed by a final slow centrifugation at 200 $\times$ g for 5 minutes. After the preparation of a standard, a known amount of the supernatant $^{51}$Cr-platelet suspension was administered to the patient by intravenous injection.

Samples of whole blood (7.5 ml) collected in EDTA were taken at 10 minutes and at 1 hour after rein-fusion of the labeled platelets, twice daily for the next 3 days, and once daily for the final 4 days. The first two samples of blood were collected at the blood bank; the additional 10 samples were obtained by the patient’s local physician and then mailed to the Mayo Clinic at the end of the eighth day, or they were obtained locally. Exactly 5 ml of each of the blood samples was placed into a plastic counting tube, and all samples were counted at once for radioactivity in a gamma spectrometer (1185 series, Nuclear Chicago). The proportion of labeled platelets remaining within the circulation after infusion (i.e., “recovery”) was calculated from the platelet activity per milliliter, extrapolated to zero time, multiplied by the estimated blood volume, and divided by the platelet $^{51}$Cr activity injected. Platelet recovery averaged 60 ± 9%.

With the use of computer-assisted least-squares analysis, a single exponent was fitted to the platelet count-rate data for determination of the platelet survival half-time. Analysis of the platelet survival by fitting a linear curve or by fitting a $\gamma$-function provided comparable results to those obtained by the above method. However, variation (expressed as the standard error of the mean) was greater when the data were analyzed by the latter two methods. In the present report, the half-time data were presented, including the 10-minute and 1-hour isotope counts on the first day; however, regardless of whether the isotope counts at 10 minutes or at 1 hour on the first day were included or excluded from the analysis (the latter because of the assumption that homogeneity of body distribution of the labeled platelets is achieved in a longer period of time), the results were not signifi-cantly different in any of the study groups.

Treatment

Dipyridamole given orally in combination with aspirin (acetylsalicylate) three times a day (each dose 75 mg of dipyridamole and 330 mg of aspirin) was ad-
ministered to 12 of the patients (11 males and one female) in group 1 who had a platelet survival half-life of less than 92 hours. The drug combination was started after the platelet survival study and was continued for 6 months until a second platelet survival study was performed. Five of the patients (all males) who remained untreated and also had two platelet survival studies 6 months apart served as controls.

Seven of the subjects (five males and two females) in group 2 who were smokers and had a platelet survival half-life of less than 92 hours agreed to discontinue smoking totally for 4 weeks, until a second platelet survival study could be performed. Discontinuation of smoking was complete in all subjects, as confirmed by close relatives, friends and companions at work. Five subjects (all males), four who continued to smoke and one subject who had a strong family history, also had two platelet survival studies and served as controls.

Results

Platelet Survival (fig. 1)

In the 50 patients of group 1, the average platelet survival half-time (± SEM) was significantly shortened, 89 ± 1.98 hours, compared with the survival half-time in the 28 control subjects of group 3, 103 ± 2.8 hours (p < 0.01, rank-sum test). In group 1, 30 patients (60%) had a platelet survival half-life shorter than 92 hours; seven of these patients were currently smoking. The shorter platelet survival half-life was found more often in patients who had both a strong family history of coronary artery disease and a history of cigarette smoking. In the 50 patients of group 1, there was no correlation between the platelet survival half-life and the degree and extent of the disease as determined arteriographically.

In the 25 subjects of group 2, who had a strong family history of coronary artery disease or a history of cigarette smoking, average platelet survival half-life was significantly shortened, to 91 ± 2.0 hours, compared with the survival half-life in the 28 control subjects of group 3, 103 ± 2.8 hours (p < 0.01, rank-sum test). The platelet survival half-life was not significantly different between the patients of group 1 and those in group 2. Among the study subjects in each of the three groups, platelet survival was not significantly different in males compared with females.

Effects of Dipyridamole and Aspirin Therapy (fig. 2)

The average platelet survival half-life of the 12 patients in group 1 who were treated with dipyridamole and aspirin was significantly prolonged, from 79 ± 1.76 hours before therapy to 89 ± 2.17 hours with therapy (p < 0.01). In contrast, the repeated platelet half-life in the five control untreated patients was not significantly different (mean variability ± 2.3%).

Effects of Discontinuation of Smoking (fig. 3)

The average platelet survival half-life of the seven subjects in group 2 who discontinued smoking was significantly prolonged, from 83 ± 1.66 hours while smoking to 92 ± 3.7 hours with discontinuation of smoking (p < 0.01). In contrast, the repeated platelet half-life measurement in the four control subjects who continued to smoke and in the control subject with a strong family history did not change significantly (mean variability ± 2.0%). In addition, in six smokers of group 2 who had a normal platelet half-life and who continued to smoke, a repeated platelet half-life measurement was not significantly different (mean variability ± 4.9%).

Figure 1. Distribution of platelet survival half-life measurements in the three groups of subjects. In each subject the absence or presence of cigarette smoking or family history is indicated. The arbitrary dashed line separates in each of the three groups, the subjects with a platelet half-life ≥ 92 hours from those with a platelet half-life < 92 hours (who are expressed as percent of the total). The solid horizontal lines denote the average platelet survival half-life.
Coronary atherosclerotic disease in the young adult is one of the major challenges faced by physicians and cardiovascular surgeons. If successful preventive measures are to be found, we must better understand the development of this premature disease. Convincing evidence indicates that cigarette smoking and strong family history of coronary artery disease play an important role in the development of coronary atherosclerotic disease in the young adult. Also, because of the suggestive role of platelets in atherogenesis, we investigated in subjects age 50 years or younger whether these two risk factors are interrelated. We also studied the activation of platelets as measured by the platelet survival time.

Platelet Survival

Platelet survival half-life was significantly decreased in our young adults who had symptomatic coronary atherosclerotic disease proved by arteriography. The findings of shortened platelet survival in patients with coronary artery disease are in accord with other recent findings. The shortened platelet survival probably reflects increased platelet consumption at the level of the coronary atherosclerotic lesions. Thus, as demonstrated by Ritchie and Harker, the increased platelet consumption is interrupted with saphenous vein coronary bypass surgery, implying that the consumption occurs largely within the coronary arteries. After coronary bypass graft surgery, Steele et al. observed a normal platelet survival.
let survival if the grafts were patent and a diminished platelet survival if the grafts were occluded.

Platelet survival in earlier smaller series of patients with atherosclerotic disease provided conflicting results, and three important points should be made. First, the shortened platelet survival in our large study group of young persons is not surprising, because coronary artery disease in the young usually indicates an accelerated atherosclerotic process; thus, one should expect platelet utilization to be greater in this group than in older patients with rather chronic disease. Second, our control patients were free of known risk factors for coronary artery disease, and therefore, are more likely to be free of subclinical coronary atherosclerosis; also, they were similar in age to patients of the study group. Third, technical considerations are important to ensure reliability of the test. Thus, after labeling, sufficient 67Cr-labeled platelets should be injected (20 μCi minimum) and documented on the initial drawn blood sample (minimum of 500 counts/min); blood samples should be carried out long enough (minimum of 7 days) and frequently enough (minimum of 10 samples) to ensure accurate determination of the platelet survival time. In determining the platelet survival time, we used three methods, including the recently recommended method based on a γ-function fitting. Although the results were comparable with the different methods of analysis, we observed significant variability (expressed as the standard error of the mean), particularly with the γ-function fitting method; such variability has also been recently reported. Therefore, we prefer to express our data in terms of half-time as assessed by least-squares analysis from a single exponent fit.

The failure of platelet survival to correlate with the extent of the disease as determined arteriographically is in accord with recent observations. This suggests that angiographic arterial narrowing does not predict the degree and extent of endothelial damage, turbulent high-velocity flow, or exposed atheromatous material, major factors implicated in platelet thrombogenesis.

The shortening in platelet survival in a significant number of apparently normal subjects who were smokers raises two important questions: The first, from a clinical standpoint, is whether the apparently normal person who is a smoker and has an increase in platelet consumption is at a higher risk of developing coronary atherosclerotic disease or, perhaps, already has subclinical disease. The second, from a basic investigational standpoint, relates to the ways in which cigarette smoking promotes the increase in platelet consumption and the development of atherosclerosis. One explanation is that the shortened platelet survival may only reflect enhanced platelet aggregation and consumption induced directly by smoking and that the platelet aggregates may then promote the arterial endothelial injury in the early stages of atherosclerosis. Another possibility is that smoking may directly induce the arterial endothelial injury and that the increased platelet consumption may then reflect the adherence or deposition of these cells to the damaged arterial sites in the process of atherosclerosis. Of the large number of toxic chemicals and effects identified in cigarette smoke, those most actively investigated are carbon monoxide leading to tissue anoxia, nicotine leading to the mobilization of catecholamines, and a tobacco glycoprotein (TGP) leading to a hypersensitivity response.

Both a strong family history of coronary artery disease and shortened platelet survival were present in a significant number of our young adults with coronary artery disease. Thus, the shortened platelet survival in two of three apparently normal persons with a strong family history is of particular interest. In these persons, the shortened platelet survival might represent subclinical disease. Little is known about how genetic factors contribute to the development and progression of coronary disease in the young. One hypothesis is that the genetic factor may contribute to a hyperactive response by the arterial wall to the endothelial injury; that is, it might induce an exaggerated proliferation of smooth muscle cells or fibroblasts or an excessive production of collagen. Alternative hypotheses are that the genetic factor may in some way lessen the resistance to the arterial endothelial damage or perhaps enhance the platelet–arterial wall interaction. Finally, although familial hyperlipidemia has a genetic basis, our study group of patients with a strong family history of coronary artery disease did not have significant evidence of familial hyperlipidemia.

Effects of Treatment

In the patients with coronary artery disease and shortened platelet survival, the interruption of platelet consumption by the use of dipyridamole and aspirin is in accord with recent findings. It appears that low-dose aspirin selectively affects the platelet by preventing generation of the proaggregating thromboxane A2, while dipyridamole interrupts platelet consumption by potentiating the effect of the platelet inhibitor prostacyclin. The platelet-suppressive action of these agents might ultimately prevent the release of platelet mitogenic factor into the vessel wall in the process of atherosclerosis. Although the direct clinical implication of these observations is uncertain, they suggest the potential for modification of the atherosclerotic process with platelet-inhibitor agents.

In the apparently normal persons who were smokers and who had shortened platelet survival, the interruption of platelet consumption after discontinuation of smoking is of interest. Mustard and Murphy made similar observations, although most of their patients already had symptomatic vascular disease. If the apparently normal person who is a smoker and has a shortened platelet survival is at risk for the development of coronary artery disease, the interruption of platelet consumption after discontinuation of smoking may be relevant. Thus, the increased platelet consumption in cigarette smokers may be a clue that establishes a link between platelets and
cigarette smoking and the development of atherosclerotic disease. The interruption of platelet consumption after discontinuation of smoking might then support the epidemiologic data that indicate a decreased risk of coronary artery disease and its complications in normal persons and patients who stop smoking.8, 9

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