Platelet Deposition at Carotid Endarterectomy Sites in Humans

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Following carotid endarterectomy, early postoperative thrombosis or late restenosis occurs in up to 20% of vessels. Both complications may be related to platelet mechanisms. To assess platelet deposition at endarterectomy sites, we injected indium-111 labelled platelets in 24 men < 30 minutes after carotid endarterectomy, with subsequent imaging 24–96 hours later. To determine if deposition decreased over time, 12 patients had follow-up studies 0.5–24 months later. For comparison, 2 control groups were studied: 1) patients with noncarotid surgery (n = 6) and 2) normal young subjects without endarterectomy and without evidence of carotid disease (n = 12). Quantitative analysis was performed using a deposition index that compared activity in operated with unoperated sites in surgical patients or activity in the right with left carotid arteries in normal subjects. Patients with recent endarterectomy had a mean deposition index of 1.7 ± 0.5 (range 1.2–3.5) compared with a similarly determined ratio of 1.1 ± 0.1 in normal subjects and 1.2 ± 0.1 in the surgical controls (both p < 0.05 vs. acute endarterectomy). At follow-up after endarterectomy, the mean deposition index decreased to 1.0 ± 0.1, documenting reduced platelet deposition over time. We conclude that the arterial injury of carotid endarterectomy results in early platelet deposition, which is no longer present in most patients who are studied late. These findings suggest a reduction in platelet thrombus formation with time and are compatible with reendothelialization of the endarterectomized surface. This model may be useful for the in vivo assessment of therapies designed to reduce platelet accumulation following endothelial injury in humans. (Stroke 1987;18:722–727)

Carotid endarterectomy involves the removal of atherosclerotic plaque with resulting exposure of a relatively large area (approximately 4–12 cm²) of underlying medial collagen and adventitia. Two complications that may be related to platelet mechanisms can occur. First, early thrombotic occlusion develops in approximately 2% of vessels.1,2 A second complication is late recurrent stenosis, which may be related to platelet release of factors that cause smooth muscle cell proliferation.3 Recurrent carotid stenosis following endarterectomy occurs relatively frequently (9–18%).4–8

The purpose of this study was to assess platelet deposition early and late following carotid endarterectomy in humans using indium-111 platelet imaging. Normal subjects without surgery, as well as subjects with surgery but no endarterectomy, served as controls.

Subjects and Methods

Patients

Twenty-four male patients, aged 47–82 years, underwent carotid endarterectomy for focal neurologic symptoms and internal carotid artery stenoses. Because of the potential benefit conferred by antithrombotic drugs in patients with transient ischemic attacks (TIAs), no attempt was made to discontinue such agents preoperatively. Preoperative and postoperative drugs are listed in Table 1. All patients received heparin (5,000–10,000 units) intraoperatively. Antithrombotic agents were given to 16 of 24 patients prior to surgery and to 10 of 24 patients early following surgery during the time of platelet imaging. Only 5 patients did not receive either preoperative or postoperative antithrombotic drugs. Eight patients had a right and 16 had a left carotid endarterectomy. Twenty-three of the 24 patients had intraoperative contrast angiography after the endarterectomy but before skin closure, which documented the absence of technical complications or stenosis of > 20% in each patient. The remaining patient did not have intraoperative arteriography.

To determine whether platelet deposition decreased over time, all subjects were requested to return for repeat study. Twelve patients were not restudied because of death (1), distance (4), refusal (5), or additional noncarotid surgery (2 patients). Twelve patients were restudied at intervals of 0.5–24 months after surgery (mean 12 ± 10 months). The interval was 0.5–2 months in 4, 8–11 months in 4, and 20–24 months in 4 patients. Nine patients were not receiving anticoagulants or platelet inhibitory drugs at the time of restudy, 2 were receiving aspirin plus dipyridamole, and 1 was receiving warfarin. None of the patients who were restudied had developed recurrent symptoms referable to the operated vessel.
Platelet Labelling and Imaging

Two control groups without carotid endarterectomy were also studied. Twelve normal subjects, aged 23–32 years, were studied. None of these subjects had TIAs or strokes, and none were receiving any medications. As a surgical control group, 6 subjects, aged 39–74 years, who had noncarotid surgery and were receiving no antithrombotic drugs were studied with immediate postoperative platelet injection and subsequent imaging. These patients were studied to gain an estimate of the platelet deposition that occurs at operative sites at which a similar amount of tissue dissection, but no endarterectomy, was performed. Five subjects had an inguinal herniorrhaphy and the sixth an exploration of the right popliteal fossa with dissection and opening of the popliteal artery but no endarterectomy. The study was approved by the University of Washington Human Subjects Review Committee, and all subjects gave informed consent.

Platelet Labelling and Imaging

Blood for platelet labelling was obtained by venipuncture immediately prior to operation in surgical patients. Platelets were labelled using a closed blood bag modification of the technique of Thakur et al as we have previously described. Since the initial description, we have changed the first (350g for 15 minutes) and second centrifugation (1300g for 15 minutes). The labelling efficiency was 44 ± 9% (mean ± SD). For acute studies in endarterectomy patients and the surgical controls, platelets were reinjected into a vein within 30 minutes of the completion of surgery, typically while the patient was in the recovery room. We chose this time for injection of labelled platelets to minimize potential platelet accumulation in extravascular tissues due to intraoperative or early postoperative blood loss. The mean injected indium-111 dose was 367 ± 33 μCi for acute endarterectomy studies, 348 ± 17 μCi for late endarterectomy studies, 314 ± 42 μCi in studies of normal control subjects, and 344 ± 14 μCi in studies of surgical controls. The mean percent of indium-111 free in the plasma (i.e., not bound to the platelets) at 24, 48, 72, and 96 hours after labelled platelet injection for all studies was 6 ± 3%, 6 ± 4%, 6 ± 3%, and 7 ± 2%, respectively. For endarterectomy patients and normal controls, anterior images of the carotid area were obtained for 100,000 counts between 24 and 96 hours after labelled platelet injection using a Sigma 410 Ohio Nuclear Gamma Camera equipped with a Model 28W08610 medium-energy parallel-hole collimator. Both the 173 and 247 keV gamma photon peaks of indium-111 were collected using a 15% window and entered into a computer in a 128 × 128 matrix (Medical Data Systems, Ann Arbor, Mich.) for quantitative analysis and on Tri-lens Polaroid film for visual analysis. For the surgical control patients, similar images were obtained of the operative site and the contralateral side.

In the patients with recent endarterectomy, imaging was performed on the day that the patient was transferred out of the surgical intensive care unit and on each day thereafter until discharge or 96 hours after labelled platelet injection. Six patients with recent endarterectomy could be imaged at 24 hours after endarterectomy, 18 at 48 hours, 15 at 72 hours, and 7 at 96 hours. For the 12 follow-up studies in patients with endarterectomy, images were obtained at 24, 48, and 72 hours after labelled platelet injection in all patients and at 96 hours in 3 patients. For the normal control subjects, images were obtained at 24, 48, 72, and 96 hours after labelled platelet injection, and for the 6 surgical control subjects, images were obtained at 24 hours in 3, at 48 hours in 4, at 72 hours in 3, and at 96 hours in 1 patient.

The platelet image data on all endarterectomy subjects was quantitatively analyzed using a deposition index (DI) that compared activity in the operated carotid artery with that of the contralateral side (Figure 1). Profiles of activity 2.9 in. wide were obtained at all imaging times through both carotid arteries using a computer program. A peak of activity corresponding to the carotid artery was apparent on all profiles. The carotid activity for each side was taken as the mean of the 4 highest adjacent pixels from the activity profile.

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**Figure 1.** Quantitative analysis of platelet deposition comparing count activity in the operated carotid with the contralateral vessel using profiles 2.9 in. wide and averaging the 4 highest pixels on each side. In this subject, who had a right carotid endarterectomy 48 hours previously, deposition index was 2.4.
DI was then calculated by dividing the counts from the operated side by the counts from the opposite, unoperated side. In patients with recent endarterectomy, the operated side always had a higher value than the opposite side. DI in normal subjects was obtained in a similar fashion by dividing activity in the right carotid artery by activity in the left. In the surgical control patients who had an inguinal herniorrhaphy, DI was calculated by comparing the operated with the unoperated side. In the surgical control patient who had a popliteal artery dissection, DI was calculated by comparing the operative site with the proximal thigh since the contralateral leg had been amputated. Since DI did not change between the serial 24- to 96-hour imaging times in any of the groups, a single mean DI was calculated for each patient or control by adding the individual DIs obtained at each imaging time and dividing by the number of images obtained. Statistical analysis was by paired and unpaired t tests using the Bonferroni correction for multiple comparisons. Data are reported as mean ± SD.

Qualitative visual analysis of each set of images in patients with endarterectomy and in both control groups was performed by an experienced observer who was unaware of any clinical data or the study sequence. Images were defined as positive if there was an area(s) of localized activity that was clearly greater than the activity in the contralateral side. Studies were defined as equivocal if there was a faint increase in platelet uptake on one side and as negative if activity on both sides appeared equal.

Results

Quantitative Results (Deposition Index)

DI for the 12 normal control subjects was 1.1 ± 0.1 (range 0.9–1.3). DI in the 6 surgical control patients was 1.2 ± 0.1 (not significantly different from normal controls). In contrast, the 24 patients studied early following endarterectomy had a DI of 1.7 ± 0.5 (range 1.2–3.5, p ≤ 0.001 vs. normal controls and p < 0.05 vs. surgical controls). Individual DIs in the 3 groups are shown in Figure 2. DI was not significantly lower in patients who received preoperative or early postoperative antithrombotic agents (1.8 ± 0.5) than in those who did not (1.5 ± 0.2).

All 12 patients studied late following endarterectomy had a decrease in DI, and the mean ratio (1.0 ± 0.1) (p < 0.001 vs. the acute study), had returned to the range seen in normal controls (Figure 3). Eleven of 12 subjects had ratios of ≤ 1.1 at late study. The single patient who had the smallest decline (1.5 to 1.3) was restudied only 1 month postoperatively.

Visual Results

The visual results were concordant with the quantitative results. By visual analysis, none of the 12 normal subjects had abnormal platelet deposition; 3 surgical control patients had positive deposition, and 3 surgical controls had equivocal or no deposition.

In contrast, of patients studied early following endarterectomy, 21 studies were positive (DI mean 1.8, range 1.2–3.5), 2 were equivocally positive (DI 1.4 and 1.5), and 1 was negative (DI 1.2) (Figures 4 and 5). The positive and equivocally positive studies had visually increased platelet uptake on the operated side in all cases. Of the 12 patients studied both early and late, all had positive studies initially. At late follow-up, none were positive, confirming the quantitative results.

Discussion

The results of this study demonstrate that platelet deposition occurs at the site of carotid endarterectomy immediately after surgery in humans. Visually positive or equivocal deposition was present in 23 of 24 patients. Our quantitative results more convincingly document platelet deposition; counts in the region of the operated carotid artery were higher than counts in the contralateral unoperated vessel in all patients, including patients with visually equivocal or negative images. Our visual results are similar to the findings of Lusby et al., who visually detected indium-111 labelled platelet deposition in 16 of 17 patients when labelled platelets were injected early after endarterectomy.

The sequence of histologic changes which occur following endarterectomy of normal vessels in animals has been defined in detail. Without intraoperative heparin, gross thrombosis occurs in all cases and is totally occlusive in approximately one half. Postoperative thrombosis in animals is reduced by the use of intraoperative heparin; only a relatively thin monolayer of confluent, flattened platelets forms immedi-
PLATELET DEPOSITION AT ENDARTERECTOMY SITES

DEPOSITION INDEX—EARLY AND LATE STUDIES

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FIGURE 3. Deposition index decreased in all subjects who had both acute and follow-up studies.

Our finding of a uniform decrease in platelet deposition in all patients studied late (0.5–24 months) after endarterectomy suggests that resolution of early mural thrombus occurs. This phenomenon has not been described previously in humans. The exact time course of the reduction in deposition cannot be determined from our data. However, the 1 patient who was restudied at only 1 month after surgery had a DI of 1.3, which was outside the range seen in most of our control patients. This patient, who had evidence of continued platelet deposition 1 month after surgery, had a stroke in the distribution of the operated vessel 1 month later.

The complications of carotid endarterectomy in humans include early occlusion and late restenosis, both of which may be platelet-related. Hertzer et al, using digital subtraction angiography, recently documented that 1.9% (5 of 262) of endarterectomized internal carotid arteries were completely occluded prior to hospital discharge and an additional 0.8% (2 of 262) were narrowed by > 30%. Both animal and human studies suggest that early occlusion is due to gross platelet-fibrin thrombosis. An additional late complication that is being increasingly recognized is recurrent stenosis, which has been clinically apparent in only 0.6–4% of patients. However, prospective noninvasive studies have documented that the recurrent stenosis rate is much higher, in the range of 9–19%. In a recent study from this institution, 17% of 145 vessels developed significant restenosis (> 50% diameter reduction) over a mean 18-month follow-up. Restenosis is often not clinically recognized since it may remain undetected until neurologic symptoms develop or because complete carotid occlusion can occur without symptoms. The histology of recurrent stenosis changes with time following endarterectomy. Lesions developing within the first 24 months have been composed primarily of exuberant myointimal hyperplasia with scarce lipid deposits, whereas later lesions are usually composed of atheromas. It has been suggested that myointimal hyperplasia and atherosclerosis may represent different phases of recurrent atherosclerosis, but this is not yet clear.

It is highly likely that platelet mechanisms contribute to carotid restenosis. In animal models, mechanical injury to the arterial wall leads to platelet adherence and gross platelet-fibrin thrombus on the denuded vessel. Smooth muscle cell proliferation, which has been attributed in part to the release of platelet-derived growth factors (PDGF) that are mitogenic for smooth muscle cells, ensues. It is possible that the magnitude

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(ANTERIOR VIEWS—48 HOURS)

FIGURE 4. Anterior indium-111 platelet images of the head and neck of a young normal control subject (left) (deposition index = 1.0) and of a patient with a recent left carotid endarterectomy (right) (deposition index = 2.1). Platelets accumulated at the endarterectomy site. Both images were obtained 48 hours after injection of labelled platelets.

FIGURE 5. Anterior indium-111 platelet image obtained 48 hours postoperatively. The liver is at the lower left of the image and the spleen at the lower right. Platelets were deposited at the endarterectomy site in the upper right of the image (deposition index = 2.0).
of the early platelet deposition response, or its failure to subside over time, correlates with restenosis. This hypothesis merits additional study. The late reduction in platelet deposition noted in the current study suggests that release of PDGF may not persist following endarterectomy; although PDGF may be important for initiating the process that results in restenosis, other mechanisms may account for the continuation of the proliferative response.

The course of platelet deposition at sites of arterial injury or endarterectomy has not been studied in detail in humans. Animal studies have documented that platelet deposition is maximal if labelled platelets are present in the circulation at the time of arterial injury, and it is therefore possible that our results underestimate the magnitude of deposition following carotid endarterectomy. In the current study, labelled platelets were not injected before endarterectomy since this would have led to extensive platelet accumulation in the surgical field and would have confounded our results. Instead, labelled platelets were injected approximately one-half hour after surgery to minimize any accumulation of platelets outside of the operated vessel in the surgical wound. Over the short time course examined in the acute studies (24–96 hours postoperatively), there was no visual or quantitative indication that platelet deposition either increased or decreased. Our acute findings are consistent with the interpretation that labelled platelets are deposited early in the endarterectomized vessel (i.e., prior to the initial imaging at 24 hours) and that there is no further net increase in platelet accumulation in the following 3 days.

The main limitation of this study is the limited resolution of gamma imaging systems, which cannot distinguish between intravascular platelet accumulation on the denuded surface due to the arterial injury and extravascular accumulation due to hemorrhage in the operative field. Additionally, although hemostasis during the procedure was meticulous, we cannot exclude the possibility that part of the labelled platelet accumulation that we detected was outside the endarterectomized vessel. However, it is unlikely that extravascular accumulation accounted for the results seen in endarterectomy patients for several reasons. First, in the animal study of endarterectomy noted above, in which labelled platelets were injected at a similar time postoperatively, there was minimal platelet activity present outside the vessel but abundant deposition on the luminal surface of the denuded vessel by postmortem scanning electron microscopy. Second, our observations in 6 subjects who did not have endarterectomies but had similar amounts of tissue dissection suggests that there is minimal platelet deposition in operated tissues when platelets are injected one-half hour after the completion of surgery. In these surgical control subjects, only faint uptake was visually detectable, and DI was significantly less than that in patients with recent endarterectomy. Another limitation was the lack of late imaging studies in many patients.

The measurement of platelet accumulation, DI, also has clear limitations since it could vary between patients due to differences in blood volume on the 2 sides or to localized uptake in the unoperated vessel. However, variations in these factors are not likely to account for the marked differences seen between the studies in patients with recent endarterectomy and the 2 control groups. Moreover, such variations do not account for the uniform finding of a reduction in platelet deposition over time since each patient served as his own control. Although the reduction in deposition over time is compatible with reendothelialization, the data offer no direct evidence. An additional limitation of this study is that the use of antithrombotic drugs was not controlled. Due to the efficacy of platelet inhibitory agents in men with TIAs and strokes, we did not feel justified in discontinuing these agents prior to surgery. Because of the wide variety of drugs and dosages used, no definitive conclusions can be reached as to the efficacy, or lack thereof, of a given antithrombotic regimen. However, it is important to note that commonly used pretreatment regimes with antiplatelet agents, in most cases aspirin, did not abolish platelet deposition in the early postoperative period.

The results indicate that the arterial injury resulting from carotid endarterectomy results in platelet deposition on the denuded arterial surface. The absence of deposition in most patients studied late documents that the injured arterial surface becomes relatively nonthrombotic, which is compatible with reendothelialization of the endarterectomized segment. This human model may be useful for direct assessment of therapies designed to limit platelet deposition following endothelial injury and will be useful in defining the relation of platelet deposition to recurrent stenosis following carotid endarterectomy.

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