PRELIMINARY NOTES

Imaging of Platelets in Right-Sided Extracardiac Conduits in Humans

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As a connection between the systemic venous ventricle and the pulmonary artery, valved Dacron extracardiac conduits have remarkably influenced the surgical approach to many complex congenital heart defects. Obstruction of the conduit, however, can reduce the long-term effectiveness of this corrective procedure. In addition to stenosis of the porcine valve, formation of thick fibrous neointima plays a major role in the pathogenesis of conduit obstruction. The purpose of this study was to determine whether platelet deposition could be demonstrated in these conduits by external imaging with In-111-labeled autologous platelets. After injection of labeled platelets either immediately after operation or on the fifth to eighth postoperative day, imaging was performed by standard procedures. Eight of nine patients had platelet accumulation in the conduit, and treatment with aspirin and dipyridamole caused no recognizable change in platelet deposition. This study demonstrates the feasibility of imaging platelet deposition in Dacron conduits and shows that the pattern of deposition varies with time.

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The use of a porcine-valved extracardiac conduit to establish continuity between the systemic venous ventricle and the pulmonary arteries in several complex forms of congenital cardiac lesions—for example, transposition of the great arteries, truncus arteriosus, or pulmonary atresia with ventricular septal defect—has allowed complete repair of conditions that would otherwise be amenable only to palliative procedures (*1–4*). The surgical results of such repair have been encouraging (*1,2,5*), but recent reports indicate that obstruction can occur in such synthetic conduits, necessitating replacement (*1,2,5–8*). The obstruction noted within 4 yr postoperatively in approximately 4% of patients (*6*) has been attributed mainly to the formation of a thick fibrous peel (or neointima) within the conduit, with or without concomitant stenosis of the porcine valve of the conduit (*1,2,6*). Autopsy observations on right-sided extracardiac conduits have shown that as early as a few days after the implantation, the conduit may be lined by a very thin layer of gray-tan material consisting primarily of platelets and fibrin, admixed with a few erythrocytes (white thrombus) (*6*). The progressive thickening of such a peel is due to an organization process occurring near the external surface of the peel in contact with the conduit and not on the internal surface in contact with the luminal blood (*6*). We have been unable to attribute any importance of mural thrombus to the development of a severely obstructive fibrous peel (*6*). Thus, the exact role of platelets in the pathogenesis of early formation of neointima is currently undefined, but the presence of platelets in the early peel has been clearly demonstrated (*6*).

Nontraumatic studies of platelet deposition in vivo in human subjects were not possible before the recent introduction of imaging procedures with autologous In-111-labeled platelets (*8–16*). Since the development of this technique, platelet deposition has been demonstrated in several vascular lesions and vascular grafts in the systemic circulation (*9–13*). Less is known about platelet-graft interaction in the central circulation, where conditions of pressure and blood flow are different.

In this study, our objective was to evaluate the feasi-

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TABLE 1. PROFILE OF PATIENTS WHO UNDERWENT IMAGING PROCEDURE

	Patient data			Operative data			Tracer studies	
Case	Age (yr), sex	Diagnosis	Preoperative platelet count ($\times 10^3$/mm^3)	Type of procedure	Type of conduit and internal diameter (mm)	Site of conduit*	Onset	Duration (hr)
1	17 M	Truncus arteriosus	189	Complete repair	Carpentier-Edwards, valved (25)	RV-PA	Immediately postoperatively	24
2	5 M	Pulmonary atresia and VSD†	223	First-stage repair	Woven Dacron, nonvalved (14)	RV-PA	Immediately postoperatively	48
3	8 M	Pulmonary atresia and VSD	236	First-stage repair without cardiopulmonary bypass	Woven Dacron, nonvalved (12)	RV-PA	Immediately postoperatively	72
4	11 M	Pulmonary atresia and VSD	282	Complete repair	Ionescu-Shiley, valved (22)	RV-PA	Immediately postoperatively	144
5	6 F	Pulmonary atresia and VSD	193	Complete repair	Low-porosity Meadox, nonvalved (18)	RV-PA	6 days postoperatively	96
6	36 F	Pulmonary atresia and VSD	107	Complete repair	Ionescu-Shiley, valved (26)	RV-PA	5 days postoperatively	120
7	15 F	Pulmonary atresia and VSD	454	Complete repair	Hancock, valved (25)	RV-PA	8 days postoperatively	120
8	5 F	Truncus arteriosus	137	Complete repair	Hancock, valved (20)	RV-PA	5 days postoperatively	120
9	39 F	Severe tetralogy of Fallot	126	Complete repair	Hancock, valved (25)	RV-PA	8 days postoperatively	120

* RV-PA = right ventricle to pulmonary artery.
† VSD = ventricular septal defect.

bility of using this radionuclide imaging procedure to localize platelet accumulation in extracardiac conduits in patients having undergone surgical repair for various congenital cardiac defects. Localization of platelets by imaging procedures was considered the necessary first step before quantitation of platelet deposition could be attempted.

MATERIAL AND METHODS

Patient population. Nine patients were studied, four male and five female (Table 1), with a mean age of 15.8 yr (range 5–39 yr). The underlying congenital cardiac anomalies for which operations were indicated were pulmonary atresia and ventricular septal defect (six patients), persistent truncus arteriosus (two patients), and severe tetralogy of Fallot (one patient). By standard surgical methods, all nine patients had the conduit placed between the right ventricle and the pulmonary artery (Fig. 1). In eight of the nine, the repair was performed using cardiopulmonary bypass; in one patient a closed technique was used, without cardiopulmonary bypass. The proximal orifice for reconstruction of the conduit is created by a vertical incision and little or no excision of ventricular muscle. Care is taken to avoid a proximal angulation of the conduit. The distal end is attached to the pulmonary artery. In seven patients, this was the first procedure for placement of a conduit; in the other two the operation was performed to replace an obstructed, previously inserted, similar conduit. The conduits were synthetic tubular grafts of corrugated woven Dacron, ranging in length from approximately 10 to 20 cm, depending on the size of the patient and the underlying cardiac defect. The internal diameter of the conduits ranged from 12 to 26 mm. Six of the nine conduits had

FIG. 1. Autopsy specimen showing conduit (arrow) that connects right ventricle and pulmonary artery.

a glutaraldehyde-fixed porcine aortic valve located close to the distal end; the other three were nonvalved conduits.*

In seven patients (Cases 1 through 7), no platelet-inhibitor drugs were given before or during the study. Two patients received a combination of platelet-inhibitor drugs (Case 8: aspirin 75 mg three times a day, with dipyridamole 25 mg twice a day; Case 9: aspirin 325 mg three times a day, with dipyridamole 75 mg three times a day). This therapy was instituted on the day before the injection of labeled platelets and continued throughout the period of study.

In four patients (Cases 1 through 4) the study was performed in the immediate postoperative period, and platelets collected preoperatively were used. In the other five patients the study was performed between 5 and 8 days after the operation, and platelets labeled on the day of the study were used. Informed consent was obtained from all patients.

Preparation of autologous In-111-labeled platelets. Autologous platelets were labeled by a technique described by Heaton and associates (*16*). For the preparation of the indium-oxine compound, In-111-chloride in dilute hydrochloric acid (about 1 mCi) was complexed to 50 μl of 8-hydroxyquinoline (oxine) in absolute alcohol (1 mg/ml) and diluted in 4 ml of a sterile solution of acid-citrate-dextrose (ACD) and saline (1:7.5). The pH was adjusted to 6.5 with 0.1 *N* sodium hydroxide solution. On the day of the study, 43 ml of whole venous blood was withdrawn through a 19-gauge needle into a syringe that contained 7 ml of modified ACD solution.† The blood was centrifuged in a sterile polypropylene tube at 180 *g* for 10 min. The supernatant platelet-rich plasma was then transferred to a 12-ml sterile polypropylene conical tube and centrifuged at 1,800 *g* for 10 min. The supernatant platelet-poor plasma was removed and saved, and the platelet pellet was resuspended in 4 ml of a sterile solution of ACD and saline by repeated suction (five to eight strokes). This suspension was again centrifuged at 1,800 *g* for 10 min, and the supernatant discarded. The resulting platelet pellet was then suspended in a solution of ACD and saline that contained In-111-oxine and was incubated at room temperature for 23 min. The In-111-oxine-platelet suspension was then centrifuged at 1,800 *g* for 10 min, the supernatant removed, and its radioactivity measured. For removal of any indium not bound to the cells, platelets were resuspended in 4 ml of a solution of platelet-poor plasma and ACD, incubated at room temperature for 7 min, and centrifuged at 1,800 *g* for 10 min. Finally, the In-111 platelet pellet was resuspended in 5.5 ml of ACD and plasma and centrifuged at 100 *g* for 5 min. From this In-111 platelet solution, 5.4 ml was carefully withdrawn in a syringe.

All In-111 platelet suspensions were prepared at room temperature under sterile conditions in a laminar-flow hood. The suspension was then injected into each patient intravenously through a 19-gauge needle. The labeling efficiency was 45–83%, platelet harvesting efficiency was 45–60%, and the mean dose of indium-111 injected was 405 μCi (range 290–483 μCi).

Radionuclide imaging. In the immediate postoperative period, imaging was performed with a portable gamma camera that was interfaced to a dedicated computer and fitted with a specially designed medium-energy parallel-hole collimator.‡ For patients studied 5 to 8 days postoperatively, a standard gamma scintillation camera‖ interfaced to a computer was used. The images were recorded on film and stored in the computer memory. Five-minute images of the anterior aspect of the chest in the anterior, LAO, and RAO positions were obtained at 4 to 24 hr after injection of autologous labeled platelets. Similarly timed images were again obtained at 24-hr intervals up to a maximum of 6 days.

Radioactivity in the conduit was calculated in three patients (Cases 5, 6, and 8) in whom the conduit was easily recognizable as an area of intense, uniform uptake. For this calculation, areas of interest were set with a light pen over the conduit and over the neighboring area of the heart (blood pool). The data were normalized for unit area (1 pixel), for body weight (10 kg), and for initial dose (300 μCi). Blood-pool background radioactivity was subtracted. No attempt was made to quantitate the actual radioactivity in the conduit.

RESULTS

In all patients studied, a well-defined image of the cardiac blood pool, spleen, and liver was seen on the imaging oscilloscope within 4 hr of injection of labeled platelets. During the 5 days of observation, the radioactivity in the spleen increased for 1–3 days and then gradually declined. The radioactivity in the liver gradually increased during the study period.

In eight patients the entire conduit or part of the conduit was visualized during part or all of the imaging period (Fig. 2). Three image patterns were noted.

Pattern 1. In four patients (Cases 5 through 8), radioactivity was seen in the entire conduit, and the distribution was uniform during the entire period of study. This pattern could be recognized in the earliest image

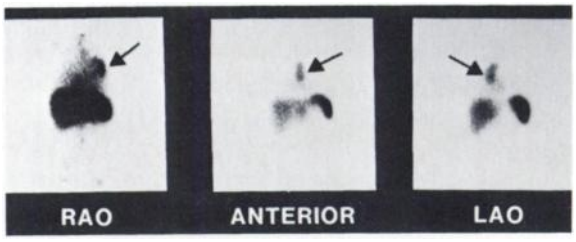

FIG. 2. Three positions used for imaging in this study, showing location of conduit (arrows) and diffuse uniform uptake. RAO = right anterior oblique; LAO = left anterior oblique.

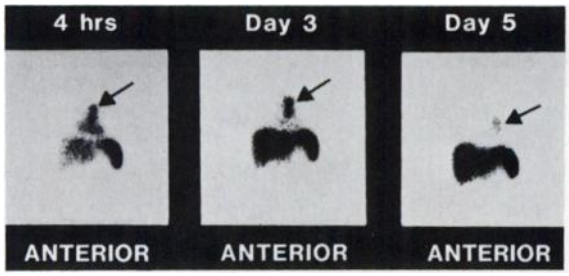

FIG. 3. (Case 8). Series of anterior images from 4 hr to 5 days after injection of autologous In-111-labeled platelets. Conduit (arrows) shows uniform platelet accumulation (Pattern 1, see text for details). Diminishing blood activity enhances appearance of conduit.

(4 hr after platelet injection) and was observed as long as 5 days later at the end of the study (Fig. 3). Radioactive platelets were deposited in the conduit early in the study and then probably remained unchanged until decay of the tracer (Fig. 4). In one patient (Case 9), uniform activity was seen only between 4 and 48 hr and not thereafter.

Pattern 2. In three patients (Cases 1, 2, and 4), focal areas of platelet accumulation were seen at various sites along the conduit. These areas corresponded approximately to the sites of surgical sutures and injury (Fig. 5).

Pattern 3. In one patient (Case 3), no radioactivity was detected in the conduit.

Of four patients studied in the immediate postoperative period, three patients (Cases 1, 2, and 4) had two or more focal areas of radioactivity (Pattern 2). The other

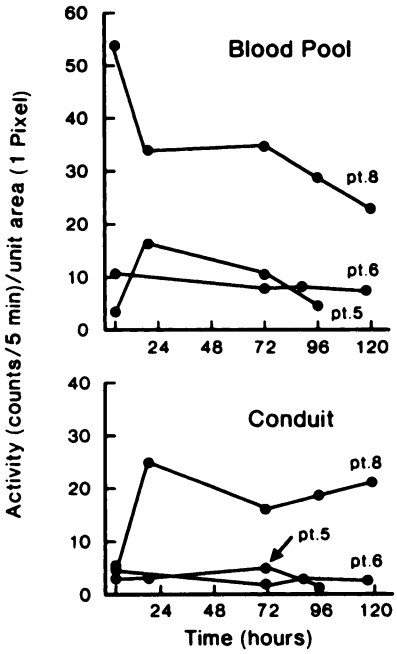

FIG. 4. Radioactivity in blood and conduit at specific times after injection of labeled platelets. Values were estimated by using computer-assisted region of interest and background subtraction. Note decline of radioactivity in blood and initial platelet deposition in conduit. Case 7 could not be analyzed for technical reasons.

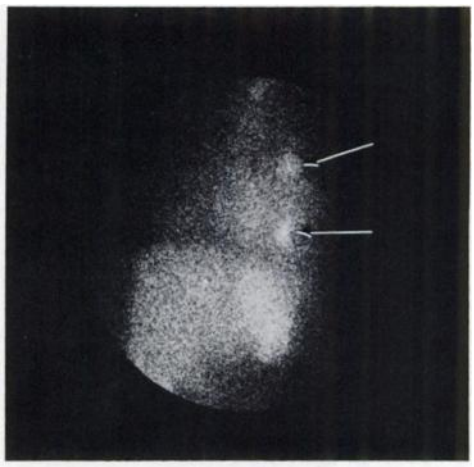

FIG. 5. (Case 2). Focal activity in conduit (arrows) (Pattern 2, see text) was characteristic in patients studied immediately after operation.

patient (Case 3) had no radioactivity in the conduit (Pattern 3). All five patients studied 5–8 days postoperatively (Cases 5 through 9) had uniform uptake in the conduit (Pattern 1); in the two patients taking platelet-inhibitor drugs (Cases 8 and 9), the pattern of radioactivity was similar to that noted in the other patients.

COMMENTS

Aortic homografts and synthetic porcine-valved extracardiac conduits are being used for reconstruction of the right-ventricular outflow tract. Tubular grafts of corrugated woven Dacron are preferred because frozen irradiated or fresh aortic homografts show calcification and obstruction more frequently.

In our experience with synthetic grafts (*6*), major obstruction has been at the valve alone in 38%, at the graft alone in 31%, and at both graft and valve in 31% of the patients undergoing reoperation for failure of the conduit.

Of interest in this study are the cases of nonvalvular obstruction, in which the stenosis is due to the formation of a fibrous peel or neointima, a phenomenon not seen in peripheral arterial grafts. In this process, a peel consisting of fibrous tissue and platelets originates at the surface in contact with the conduit and progressively thickens. We believe that, through fenestration in the neointima, luminal blood flow dissects portions of the neointima away from the surface of the conduit; this process results in blind channels into which blood can enter. Commonly, stasis of blood in these channels leads to formation of a thrombus, which then becomes organized. Recurrent separation of the peel from the conduit allows progressive fibrous thickening. Perhaps the rhythmic, accordionlike motion of the corrugated conduit contributes not only to the initial fenestration but also to the loosening of the peel from the conduit. In addition, the Dacron weave of the conduit is deliberately

made tight and nonporous for hemostatic reasons. Because active fibroblasts cannot easily penetrate the small interstices of the Dacron weave, formation of fibrous anchors between the conduit and the peel may be hindered. Platelets have been shown to participate in this mechanism of formation and organization of a thrombus, and conceivably could be considered a convenient marker for formation of a neointima.

The method of In-111 labeling of platelets developed by Thakur and associates (*8*) provides a new tool for the in vivo kinetic study of platelet deposition on vascular grafts by external imaging. Several studies in animals and humans have demonstrated the potential use of imaging with In-111-labeled autologous platelets in the diagnosis of thrombosis of coronary artery bypass grafts (*9,10*), left-ventricular thrombi (*13*), infective endocarditis (*15*), and systemic arterial and venous thrombi (*11*). Platelet labeling has also been used for assessing the thrombogenicity of prosthetic grafts in humans and animals (*9,10*). Data obtained in systemic arterial grafts are not necessarily applicable to cardiac conduits because of the difference in blood-flow and blood-pressure characteristics between pulmonary and systemic circulations. Patients with prosthetic arterial grafts have predominant platelet activation rather than fibrinogen activation, a factor that makes observations on platelet deposition particularly important.

Our in vivo study demonstrated platelet accumulation in extracardiac conduits. In right-sided conduits this is an interesting observation, especially because of the increasing evidence of malfunction of such conduits and the observation that platelets may play a role in its pathogenesis (*6*).

Currently used arterial grafts are thrombogenic and have a minimal diameter limit (4 mm) because of the associated risk of occlusion. Thrombosis and distal embolization are recognized complications and limit the use of these vascular grafts. The conduits used range from 10 to 26 mm (i.d.) and thus are similar to those used in iliofemoral bypass grafts. Although pulmonary emboli were not seen in our study, the resolution of this imaging technique may be inadequate for demonstrating small infarcts or emboli, and the possibility of such sequelae cannot be dismissed.

The relationship between early platelet deposition and late graft occlusion or embolization has not yet been established for the conduits or other arterial grafts. Future longitudinal studies may provide an answer. The finding of diffuse uptake of labeled platelets in arterial iliofemoral Dacron grafts more than 1 yr after injection of the platelets suggests, however, that platelet deposition similar to that noted in the early postoperative period could also occur in old conduits. Thus, platelet deposition may simply reflect the lack of endothelialization on those Dacron grafts, as shown by autopsy studies.

Platelet uptake seems to be a normal occurrence in Dacron prostheses and by itself may have little diagnostic or prognostic value. In our experience, however, quantitation of platelet uptake at peripheral arterial graft sites has shown that the uptake is generally less when antiplatelet drugs of proven effectiveness are given. For the cardiac conduits, satisfactory quantitation has not been achieved. Our studies, as illustrated in Fig. 4, yielded relative figures that preclude comparisons among patients and allow only limited comparison within the same patient in repeated studies. For the latter, estimation of depth, size of conduit (surface area), and tissue absorption must be considered.

The finding of no demonstrable platelet deposition in one patient necessitates further consideration. The prosthetic material was similar to that used in the other patients, yet no deposition was seen during 5 days of observation. Ritchie and associates (*13*) recently described five patients with aortofemoral or iliofemoral Dacron arterial grafts. They observed diffuse platelet deposition in four patients and none in one—findings similar to ours. No explanation for the absence of platelet deposition was readily apparent. Both faulty platelet labeling and graft occlusion can be excluded as causes in our study.

In our Cases 8 and 9, aspirin and dipyridamole did not appreciably inhibit platelet deposition in the conduit, as judged from images. Although the number of observations is small, our findings correspond to those of Ritchie and associates (*13*) with iliofemoral Dacron grafts. Both of these observations contrast with the findings in dogs reported by Fuster and associates (*10*), who used semiquantitative assessments; platelet activity was decreased in saphenous-vein bypass grafts during treatment with aspirin and dipyridamole. Minor differences in platelet deposition, however, may be imperceptible by imaging, and more sensitive procedures such as quantitation of actual radioactivity present per unit area of conduit may still disclose differences in treated patients.

Three patients studied in the immediate postoperative period had focal platelet accumulation, which most likely represented formation of thrombi at suture sites or sites of myocardial damage and perhaps also hemorrhage. Anesthetic agents and drugs remaining in the blood may also influence platelet deposition when studies are performed in the immediate postoperative period. Thus, platelets in this early model may not function normally.

This study shows that platelet deposition in conduits can be demonstrated by an imaging technique that uses In-111-labeled platelets. This model seems to have limited usefulness in studying the effect of platelet-inhibitor drugs on platelet deposition and perhaps on the occlusion of conduits when only imaging is performed. There is a possibility, as yet not fully explored for cardiac conduits, that quantitation of platelet deposition may give more relevant results. Because too many uncon-

trolled factors are present in the immediate postoperative study, the later injection period promises to be a better model for quantitation.

FOOTNOTES

* Hancock Laboratories.
† E. R. Squibb & Sons, Princeton, NJ.
‡ Cardiac Medical Systems Corporation, Northbrook, IL.
‖ Searle Radiographics, Inc., Des Plaines, IL.

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