Indium-111 Oxine Platelet Survival in Dogs: Effect of Doxorubicin and Dacron Grafts

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The cytotoxic chemotherapeutic agent doxorubicin was found to acutely decrease platelet survival times in beagle dogs as determined using platelets labeled with [^{111}In]oxine. Recovery was rapid, with platelet survival times returning to normal within 3 wk of cessation of treatment. Doxorubicin (1.5 mg/kg i.v. every 3 wk for a total of four doses) also delayed the return of platelet survival times to normal when given to beagle dogs following placement of thoracoabdominal aortic Dacron grafts. It is not known whether or not this may have clinical significance.

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Doxorubicin is a cytotoxic chemotherapeutic agent used in the treatment of many neoplasms (*1*). Neoplasms often involve an elderly population, one which is also prone to atherosclerotic peripheral vascular disease. When both diseases occur simultaneously, it may be necessary to incorporate synthetic arterial grafts in individuals who are on antineoplastic drugs. Incorporation of the graft involves infiltration of the synthetic matrix with neointima and progressive coating of the inner surface with endothelial tissue. Platelet survival times have been reported to correlate well with the degree of endotheliazation (*2, 3*). Doxorubicin, however, is taken up by platelets and may impart injury to these fragile cells (*2*).

The following study was designed to investigate the effect of doxorubicin alone and together with vascular reconstruction on platelet survival times determined with the use of indium-111 (^{111}In) oxine in an animal model. The thoracoabdominal aorta was replaced with a bypass graft of woven Dacron in beagle dogs and platelet survival times were followed periodically.

MATERIALS AND METHODS

Four groups of adult beagle dogs of both sexes were used. Platelet survival times were determined for each animal prior to entering a treatment regimen. These pretreatment values are reported as time zero values. Additional platelet survival times were determined for each animal corresponding to ~7 days, 30 days, 90 days, and at 90-day intervals thereafter postinitiation of any form of treatment (surgery or drug administration). Treatment groups were followed for at least 1 yr.

Platelet survival times were determined using [^{111}In]oxine labeled autologous platelets as described in detail elsewhere (*4*). Platelets were isolated by differential centrifugation, suspended in isotonic saline, mixed at room temperature with about 100 μCi of [^{111}In]oxine* and allowed to stand at room temperature for 15–30 min. After washing and resuspension in autologous plasma, the platelets were reinjected into the animal. Blood samples were taken at 0.5-, 2-, 24-, and at 24-hr intervals with the final sample taken 96 hr postinjection. Radioactivity in the samples was counted in a gamma counter† optimized for counting ^{111}In.

Values were normalized to the 0.5-hr sample and mean platelet survival times were calculated as recommended by the International Committee on Standardization in Hematology using the multi-hit model for platelet disappearance from circulation (*5*) (Fig. 1).

Six to nine dogs were assigned to each treatment group. Group 1 consisted of a control group of animals for which platelet survival times were determined at intervals corresponding to those of the treatment groups. Group 2 received a treatment regimen of doxorubicin (1.5 mg/kg i.v. on Day 1 and every 3 wk thereafter for a total of four doses). Group 3 underwent surgery for placement of thoracoabdominal aortic bypass grafts of woven Dacron, as described by Clagett et al. on Day 1 (*2*). The animals in Group 4 also received an aortic bypass graft on Day 1 and 90 days postsurgery a treatment regimen of doxorubicin (1.5 mg/kg i.v. on Day 90 and every 3 wk thereafter for a total of four doses) was initiated.

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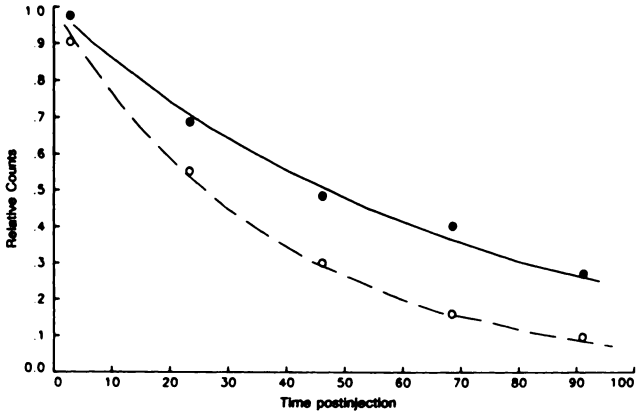

FIGURE 1
Typical platelet survival curves for an animal before graft installation (●–●) and 7 days after surgery (○–○). Curves were fitted according to a multi-hit model

The dogs that underwent replacement of the thoracoabdominal aorta received grafts of woven Dacron which were ~10 mm in diam and 28–36 cm in length. The proximal anastomosis was end-to-side at the level of the inferior pulmonary vein. The aortic prosthesis was routed behind the left hemidiaphragm to the retroperitoneal site of the distal end-to-side aortic anastomosis below the renal arteries. The thoracic aorta was ligated just below the proximal anastomosis with an umbilical tape, thus shunting all aortic blood through the prosthesis. Anesthesia was induced with sodium pentothal and mainained with halothane. All animals received ampicillin pre- and postoperatively for prophylaxis against infection.

Statistical methods

Within the control group and each treatment group, analysis was done on the mean platelet survival times (hr) of animals grouped by days into the study. Day 0 identifies a response as being pretreatment. The sample means are point estimates of population means which were estimated by 95% confidence intervals based on the t-distribution. No pooling was done here concerning variance estimates. Within each treatment group, comparison was made between the pretreatment group and each day group by a paired t-test with each dog serving as his own control. This tests the null hypothesis of no treatment effect at a particular day. Under the assumption of independent errors, the different scores of the paired t-test have a variance which is twice the within-dog variance. A one-way ANOVA by dog in the control group gives an independent estimate of this variance. Each paired t-test was based on the pooled variance estimate, accomplished in the standard way. Since the group sizes were not large, it was felt that the attendant increase in degrees of freedom afforded by this modified paired t-test, was necessary to give sufficient power to the test.

RESULTS

There was considerable animal-to-animal variability in platelet survival times, as evidenced by the size of the 95% confidence interval for the mean in the control group (Fig. 2). However, platelet survival times remained relatively constant over a 270-day period for each animal, resulting in little change in the control group means over the 270-day period. Methodology for determining platelet survival times and variability in the analysis have been discussed in detail elsewhere (*4*).

Doxorubicin was found to dramatically reduce platelet survival in dogs within 1 wk of initiation of treatment (Fig. 2, Group 2). Survival times were significantly different from pretreatment levels at 1 wk and 4 wk postinitiation of treatment. Significant thrombocytopenia did not develop during drug treatment ($p < 0.05$, paired t-test, data not shown). Recovery was rapid with platelet kinetics returning to normal within 3 wk of cessation of treatment.

Dacron graft placement resulted in greatly reduced platelet survival times with slow recovery over a period of months (Fig. 3, Group 3). Administration of doxorubicin 90 days after graft installation resulted in delayed recovery of platelet kinetics (Fig. 3, Group 4).

DISCUSSION

The acute effects of doxorubicin on platelet kinetics were unanticipated (Fig. 2). Platelet function, as measured by a variety of in vitro tests, is affected by a number of drugs. The anti-tumor drugs doxorubicin and daunomycin are structural congeners and share some clinical and toxic properties. Both are concentrated by platelets relative to plasma at doses used clinically (*6*).

Doxorubicin has been shown to increase human platelet lipid peroxidation, presumably altering membrane unsatu-

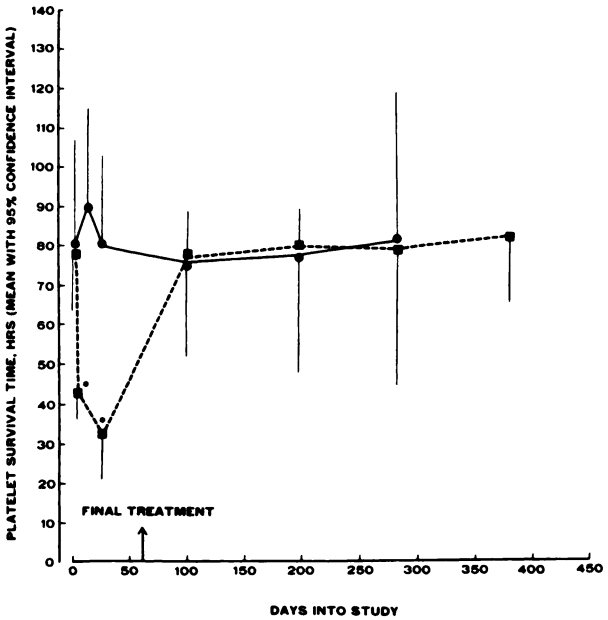

FIGURE 2
Change in platelet survival with time. Group 1 (n = 6)—control, Group 2 (n = 7)—doxorubicin treated. (●) Group 1; (■) Group 2; (*) $p < 0.025$

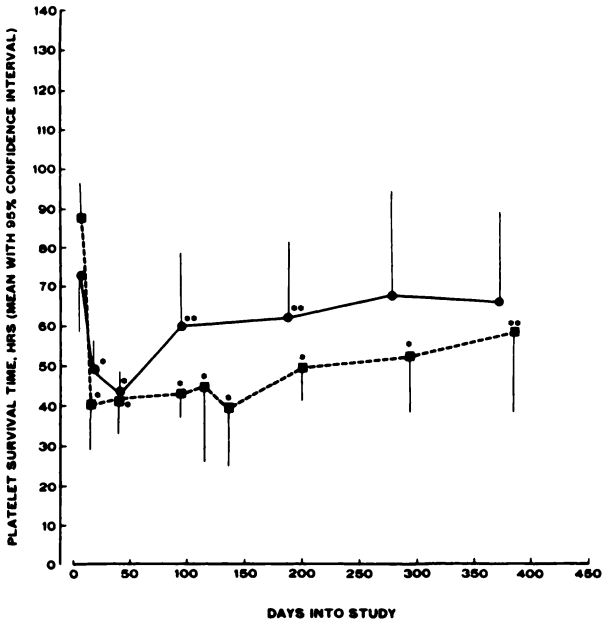

FIGURE 3
Change in platelet survival with time. Group 3 (n = 7)—graft installation alone, Group 4 (n = 8)—graft installation followed by doxorubicin treatment 90 days postsurgery. (●) Group 3; (■) Group 4; (*) $p < 0.025$; (**)$p < 0.100$

rated fatty acids *(7)*. The effect of lipid peroxidation on in vivo platelet function has not been evaluated. Daunorubicin has been shown to inhibit collagen and thrombin-induced platelet aggregation in a concentration dependent manner in vitro *(8)*.

The effects seen in this study could result from a broad range of possible actions of doxorubicin. Whether the alteration of platelet kinetics is a direct effect on the platelet, a possibility suggested by in vitro studies, or is the result of alterations in the body's platelet clearance mechanisms is not known. Further research is necessary in order to provide a sound explanation for this observation.

Clagett et al. *(2)* found that platelet kinetics in dogs with aortic thoracoaodominal Dacron grafts did not return to normal until 18 mo after surgery. Our results indicate a somewhat more rapid recovery of platelet kinetics (Fig. 3). The difference in the time required for recovery in our group of dogs, as compared to the previously reported results, is possibly due to the difference in the population of dogs used or differences in methodology.

Initiation of the doxorubicin treatment regimen (1.5 mg/kg i.v. every 3 wk for a total of four doses) 90 days after placement of the graft was intended to give an idea of the effect of the drug on graft incorporation. At 90 days postsurgery the animal has overcome the acute effects of the surgery, but the graft apparently has not become fully infiltrated by neointima as evidenced by a platelet survival time that is still reduced. Interpretation of the results (Fig. 3) must take into account the acute effect of doxorubicin on platelet kinetics. Initiation of the drug treatment regimen at 90 days postsurgery and consideration of the effects of doxorubicin on platelet kinetics prevents statistical comparison of platelet survival times prior to 180 days. However, the platelet survival times remain depressed for 288 days (Fig. 3), which is long past the time required for recovery from the acute effects of doxorubicin. It appears that doxorubicin may delay the rate of recovery of platelet kinetics to normal following placement of a dacron graft with recovery being incomplete at 288 days postsurgery while animals receiving grafts, but not exposed to doxorubicin, recovered by 273 days (Fig. 3). Of note, the platelet survival times in Group 4 (Fig. 3) are apparently not affected by the administration of doxorubicin as greatly as Group 2 (Fig. 2). This may be a reflection that platelet survival times are already depressed to a level by the surgery and the administered doses of doxorubicin may not further actuely depress the platelet survival times significantly.

The data presented here demonstrate that doxorubicin has an acute effect on platelet survival in vivo which is readily reversible upon cessation of drug treatment. The drug was also shown to delay return of platelet survival times to pre-treatment levels when treatment was initiated 90 days after graft placement. This indicates that the drug may, in some manner, delay the development of neointimal layer within the graft. The delayed recovery was, however, not dramatically different from the surgery only group.

In conclusion, the finding that doxorubicin dramatically alters platelet kinetics in vivo in the absence of thrombocytopenia indicates that the drug has acute effects on the platelet itself or on platelet removal mechanisms. The potential ability of doxorubicin to delay Dacro graft incorporation, as indicated by delayed recovery of platelet survival times, is not well understood. Further work is needed in order to determine how seriously the results noted here affect the clinical situation.

FOOTNOTES

*Medi-Physics, Inc., Richmond, CA.
†Beckman 4000, Beckman Instruments Inc., Fullerton, CA.

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